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THE EFFECT OF MONOCHROMATIC ULTRA-VIOLET LIGHT OF MEASURED INTENSITIES ON BEHAVIOR OF PLANT CELLS[1]

PRELIMINARY REPORT

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INTRODUCTION AND DISCUSSION OF PREVIOUS WORK

The effect of ultra-violet radiation on plants has been the subject of numerous investigations, but most of the results obtained are either indefinite or contradictory. Hardly anything is yet known about the physical and chemical reactions involved. To make any progress in this direction, that is, to seek a physical and chemical explanation for the action of ultra-violet radiation on living cells, it would be essential to accumulate exact quantitative data on the subject. The lack of quantitative measurements of the spectral qualities of the source of radiation is notably the weakest point in most of the investigations, and therefore it becomes almost impossible to correlate or interpret the results obtained.

According to the fundamental law of photochemistry (Grotthuss-Draper Photochemical Absorption Law) only rays which are absorbed are effective in producing chemical changes. However, not all absorbed energy has to result in chemical reactions,

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since radiation may be transformed into another form of radiant energy or produce a change in the energy content of the molecule. According to Einstein's concept of quantum absorption, the occurrence of photochemical reactions is due to the absorption of quanta of radiation, each single molecule requiring one quantum of a frequency characteristic of the absorbing molecule. This concept, based on parallelism with the photoelectric effect, indicates a more or less specific action of different quanta. A quantum of a given energy value can be expressed as a particular frequency or wave length. Of course, one should not forget that the absorption of radiant energy by a molecule is much more complex than it is in an atom and that Einstein's concept applies only to the primary stage of a photochemical reaction. Secondary chemical processes initiated by the primary stage will often be entirely independent from the light action.

It is clear, however, that the use of monochromatic light will help to explain many of the essential factors in photochemical and photobiological investigations.

The necessity of confining one's self to the study of the action of narrow spectral regions in the ultra-violet becomes especially clear if one considers the nature of absorption bands in this part of the spectrum. Ribaud ('13), who studied and compared absorption bands of different gases, liquids, and solids in different spectral regions, came to the conclusion that the width of the absorption bands decreases continuously on going from the infra-red toward the ultra-violet end of the spectrum and is nearly proportional to the wave-length maximum. It is also clear that as long as the absorption of radiant energy involves a given quantum per absorbing molecule the number of quanta or the intensity of absorbed radiant energy must be determined. The Bunsen-Roscoe reciprocity law of photochemistry states that when the product of intensity and exposure time is constant a constant photochemical reaction results. With some modifications the law holds for most of the chemical compounds tested.

As far as the biological action of radiant energy is concerned, there is no reason to believe that the laws of photochemistry are not applicable, even though the situation be much more

complex. As a matter of fact, several of the photochemical laws have been tested for biological objects. The correlation between the absorption spectrum of chlorophyll and the rate at which carbon dioxide is decomposed by the plant was studied as early as 1875 by Timiriazev ('75). Gates ('30), using accurate quantitative measurements, found that the absorption curve of bacteria corresponded strikingly to the curve obtained for the lethal action of different wave lengths and intensities of the incident energy.

Verhoeff and Bell ('16), in their investigations on the harmful effect of ultra-violet radiation on the cornea of the eye, found that the time of exposure necessary to produce symptoms of injury is inversely proportional to the intensity of radiation of the effective ray. Similar results were obtained by Hill and Eidenow ('23) and Weinstein ('30) with paramecia, and Barr and Bovie ('23) with amoebae. Coblentz and Fulton ('24) emphasized the fact that longer exposures do not fully compensate for decreased intensity. An intensity reduction to 1/50 required an increase of $\times$ 75 in the exposure time to produce a comparable reaction on bacteria. Gates ('29a) tested the validity of this Bunsen-Roscoe law by the killing effect of ultra-violet radiation on bacteria. He worked with monochromatic light and, measuring intensities by means of a sensitive thermopile, found that the law does not hold strictly, especially with young and metabolically and genetically active bacteria, although it is fairly accurate if small differences in intensities are used.

The results obtained by Verhoeff and Bell ('16), Bovie ('16), Barr and Bovie ('23), and Weinstein ('30) indicate that within certain limits, the same total exposure is required to produce the effect when the radiation is interrupted for short intervals as when it is continuous.

In the light of the preceding discussion it becomes evident that the quality and quantity of light play important parts in the effect it will produce on living matter.

The literature on the action of ultra-violet radiation on plants has been reviewed by Eltinge ('28), Arthur and Newell ('29), and Fuller ('31). As emphasized by Fuller, the fact that sources of radiation of unknown spectral quality have been used in

most investigations makes it almost impossible to compare the results obtained by various workers. He also emphasized the fact that in biological effects radiation from artificial sources of light, such as mercury vapor arcs, can by no means be compared with the radiation of the sun.

To eliminate some of the uncertainty about the spectral aspects of the source of radiation, a number of workers have used filters of various makes to limit the radiation to certain parts of the spectrum. However, unless spectrographs of the transmission of light through the filter are given one cannot be certain about the quality of the spectrum, since commercial mercury vapor arcs vary in this respect according to make, the length of time they have been used, etc. Furthermore, the use of selective filters introduces a number of complications due to the partial absorption of spectral lines other than those eliminated. The relative intensities of the different parts of the spectrum are thus distorted, and one does not know whether the effect produced by interposing a filter is due to the elimination of a certain spectral region or to the weakening of the intensity of the wave lengths transmitted. Besides, spectrographs are usually taken by interposing the filter between the spectroscope and the source of light placed close to the filter, while the objects during irradiation are placed at quite a distance from the source, sometimes as much as 100 inches. Henri (cited by Taylor, '31) claimed to have shown that a strict relationship exists between the infra-red and ultra-violet in their photochemical action. Reiter and Garbor ('28) claimed to have established an antagonistic relationship between the action of two different bands in the ultra-violet spectrum on cell division.

The only way then to get a clear picture of the action of ultra-violet light on organisms would be to use monochromatic light of measured intensities, so that the actual energy falling on the object under investigation could be definitely determined. It is true that monochromatic light does not occur in the natural surroundings of the plant, and therefore cannot be regarded as a normal environmental factor. However, the selective absorption of light by the organic substances of the plant and the fact that it affords the only accurate means of determining the

quantity and quality of light make it advisable to investigate, first, the biological action of monochromatic light and, later on, to synthesize the results.

Several investigators have exposed their objects to radiation passed through a quartz spectrograph. Ward ('93) was the first to use this method for the study of the bactericidal action of light. He was followed by a number of workers.

Hertel ('05) seems to be the first who fully recognized the importance of quantitative measurements of the intensities of monochromatic light used for biological studies. Using a quartz prism and lenses, he constructed a monochromator similar to those used in ultra-violet microscopy. He determined the relative intensities of the lines by means of a thermopile and varied the intensity by regulating the amperage of the metallic arc. He used four lines of the ultra-violet part of the spectrum and studied their effects on paramecia, diatoms, *Oscillaria*, and *Elodea*. He found that the line 2800 Å was the most powerful in its destructive action on cells, and noticed that not only was the streaming in the cells of *Elodea* retarded by the light but also that the cells finally died.

Schulze ('09) devoted himself to the study of the effect of the powerful line of 2800 Å of the magnesium spark. As objects he used cells of *Spirogyra*, *Nitella*, *Vallisneria*, and *Elodea*, root hairs of *Hydrocharis*, anther hairs of *Tradescantia*, and hyphae of *Mucor*. He employed a monochromator similar to that used by Hertel and focused the rays by means of quartz lenses on the stage of the microscope. The intensity was varied by means of regulating the amperage across a magnesium spark. He found that at certain intensities small vacuoles appear in the cells, that protoplasmic streaming is retarded, and that longer exposures result in death of the cells. The growth of hyphae of *Mucor* and the cell division in *Tradescantia* were retarded. Even when using relatively small intensities he was unable to detect stimulation. By means of microphotographs he showed that the cuticle and epidermis strongly absorb the ultra-violet of this frequency. Parenchyma tissue, phloem, and young cambium were quite transparent to the light, whereas xylem again absorbed it rather strongly. As far as the different parts of the cell were

concerned, he showed that the strongest absorption was in the middle lamella. Strong absorption was also shown by the nuclei and chromosomes. Unfortunately it is impossible to ascertain the exact intensities used in his experiments.

Frank and Gurwitsch ('27), in trying to discover the cause of the so-called mitogenetic radiation which they claimed is emitted by embryonic tissue, used a small quartz spectrograph to determine the physical nature of the radiation. They believed that the wave lengths of 1930–2370 Å at one-minute exposure produced a stimulating effect on the cell division of the root of the onion similar to that produced by mitogenetic rays.

Reiter and Garbor ('28), in their extensive study on mitogenetic rays, employed a specially constructed spectrograph which permitted them to combine at one focal point several wave lengths. By using a number of arcs and sparks to obtain a large number of lines in the ultra-violet part of the spectrum, they found that the line 3400 Å, and to a lesser degree, line 2800 Å produce a stimulating effect on cell division in the root of the onion, eggs and larvae of the frog and salamander, and sarcoma tissue of the rat. This was evident only at relatively low intensities, whereas at higher intensities the same rays were destructive. If the spectral region of 2900–3200 Å was added to the radiation of the line 3400 Å, the stimulating as well as destructive action of the radiation disappeared. Frank ('29) disagreed with Reiter and Garbor as to the wave length involved in mitogenetic radiation. In rechecking his earlier observations he found that the spectral region between 2000 and 2400 Å is effective. He even claims to have obtained a mitogenetic stimulation of yeast in this region by passing the radiation from that of a biological source through a powerful quartz spectrograph. Neither Reiter and Garbor nor Frank gave the measurements for the intensities employed.

Recently Gates ('29a, '29b, '30) published a series of investigations on the bactericidal action of ultra-violet light. Using monochromatic light from a specially constructed powerful monochromator and measuring the intensity of the incident radiation in absolute energy units by means of a thermopile, he studied the lethal effects of ten lines of the mercury vapor

spectrum on *Bacillus coli, B. communis,* and *Staphylococcus aureus.* These papers have probably contributed more to the clarifying of the topic under discussion than all the rest of the investigations taken together and demonstrated the value of the use of monochromatic light of measured intensities in the study of biological objects. In the first paper of the series Gates determined the curves of bactericidal action for each wave length studied. He found that with all the different wave lengths the reactions followed similar curves, but occurred, at each wave length, at a different energy level. In his second paper he studied the effect of various environmental factors on the bactericidal action of ultra-violet and determined the wave length limits of the action as being between 3130 and 2250 Å, although the lower limit could not be definitely ascertained. In the third paper the absorption curves of the body of the bacteria were determined and correlated with the curve of incident energy instrumental in the bactericidal action. Although some minor differences are evident in the curves, they form in general a reciprocal of each other. He proved that the belief that the shorter the wave-length the greater the bactericidal action of ultra-violet is erroneous, and that a striking maximum of effectiveness exists between 2600 and 2700 Å. Having thus accumulated quantitative data on the subject, he promises a discussion of the photochemical action of the radiation in his next paper.

Weinstein ('30), also using similar quantitative measurements and focusing monochromatic light on the stage of a microscope, made observations of the effect of five wave lengths of the ultra-violet part of the spectrum on *Paramecium micromultinucleatum.* The line of 2650 Å was found to be most effective in killing paramecia.

Marshall and Knudson ('30), by means of similar methods, studied the effect of monochromatic light on the formation of vitamin D from ergosterol. They found that the rate of production of the vitamin is directly proportional to the number of light quanta absorbed by ergosterol. The reaction was found to be independent of the wave length within the absorption region of ergosterol.

APPARATUS USED

To be able to obtain quantitative data on the action of ultraviolet radiation on plant cells a Bausch and Lomb quartz monochromator was used. The instrument is of the constant deviation type and calibrated for the wave lengths from 2000 to 8000 Å. The intensity of the radiation was measured by means of a Coblentz bismuth-silver thermopile adapted by the B. & L. Optical Company for their monochromator. The thermopile is mounted on an adapter in such a manner that it can be lowered in front of the slit when intensity measurements are taken and raised above the slit to permit the light to pass into the space. The relative positions of the thermopile (b) in its adapter (c) can be seen by comparing figs. 1 and 3, pl. 42. The metal box containing the thermopile and supplied with a quartz window was sealed air-tight and evacuated through an outlet (d). It was found that, although it was difficult to maintain a high vacuum for long periods, one could get a relatively constant partial vacuum by evacuating the box while measurements were taken. An oil vacuum pump was used for evacuation.

The thermopile was connected with a Type HS Leeds and Northrup galvanometer having a sensitivity of 20 mm. per μv. and an internal resistance of 16.8 ohms. Since the resistance of the thermopile was found to be equal to the critical damping resistance of the galvanometer (7.5 ohms) no additional resistances were placed in the circuit. The deflections were read through a telescope at 2 meters scale distance.

The thermopile was calibrated in absolute energy units (mechanical equivalent) against a Bureau of Standards carbon filament incandescent lamp No. C-109. The reaction of the thermopile was found to be linear, and a 1 mm. deflection on the galvanometer corresponded to a light energy of 7.22 erg/mm.^{2}/second. The readings of the intensity of the different spectral lines taken at various times were quite constant, varying within the limit of 5 per cent.

As a source of light, at first a horizontal air-cooled quartz mercury vapor arc of the Burdick Cabinet Company was tried but proved to be of insufficient intensity for monochromatic work. A vertical water-cooled Burdick arc (type W-910) operated

at 55 volts and 4.3 amperes was used in most of the experiments. It was placed at a distance of 4 cm. from the entrance slit of the monochromator. This lamp is supplied with a filter of water 1 cm. deep, so that most of the infra-red radiation is probably eliminated.

The distribution of the spectral lines in the ultra-violet region and their relative intensities as measured by the thermopile are represented in fig. 1.

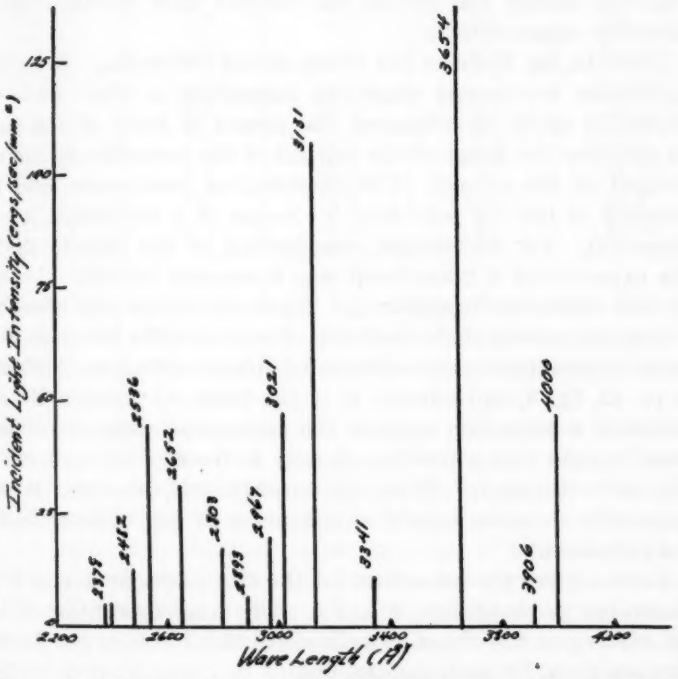

Fig. 1. Distribution and relative intensities of the spectral lines of a Burdick water-cooled mercury arc.

The widths of the entrance and exit slits of the monochromator were kept constant at 0.2 mm., representing an area of 2 mm.[2]

Considerable difficulties were experienced in so adjusting the objects studied that they could be examined by a microscope and the incident energy could be determined by the thermopile. Even at a distance of 1 cm. from the exit slit of the monochro-

mator the rays are dispersed to a considerable extent. As illustrated in pl. 42, figs. 1 and 2, a metallic tube (e) supported by an adjustable stand (g) was placed in the path of the ray passing from the monochromator (j). By means of a quartz lens (f) the image of the exit slit of the monochromator was reproduced on a second slit just behind the thermopile (b). One end of the tube was shaped in such a manner that the whole thermopile adapter (c), including the thermopile and the adjustable slit, could be removed during the raying and slipped into position during intensity measurements.

Plate 42, fig. 2, shows the set-up during the raying. A vertical adjustable microscope stage (h) supporting a slide with the object (i) under investigation was placed in front of the tube. In this way the image of the exit slit of the monochromator was focused on the subject. The observations were made, and the focusing of the ray was done by means of a horizontal microscope (a). For microscopic examination of the objects during the experiments a micro-lamp was interposed between the lens (f) and the monochromator (j). Since the quartz lens absorbed a large percentage of the radiation of wave lengths below 2536 Å, some of the experiments were carried out in a set-up as illustrated in pl. 42, fig. 3, and referred to in the tables as position B. By means of a projection built on the microscope stage the objects were brought into a position directly in front of the exit slit of the monochromator. With this arrangement, however, it was impossible to make careful examination of the objects during the experiments.

Table 1 gives the intensities for the two positions A and B as illustrated in pl. 42, figs. 2 and 3. The total intensities of the light falling on the object at a slit width of 0.2 mm. or per 2 mm.^{2} area are given for each spectral line.

Selection of Objects and Procedure

The selection of objects was limited, due to the necessity of having them exposed to a small area of light (2 mm.^{2}). Since ultra-violet radiation does not penetrate deeply into plant tissue and is known to be absorbed readily by single layers of cells, objects of single-cell thickness had to be selected. It was also im-

TABLE I

INTENSITIES OF LIGHT PER 2 SQ. MM. IN POSITIONS A AND B

Wave lengths	Intensities for position A (pl. 42, figs. 1–2)		Intensities for position B (pl. 42, fig. 3)	
Å	galv. defl. mm.	ergs/sec/2 mm.²	galv. defl. mm.	ergs/sec/2 mm.²
3663.27 3662.87 3654.83 3650.15	24.5	196.0	36.0	280.0
3341.48	1.5	12.0	2.8	22.4
3131.84 3131.56 3125.60	10.5	84.0	26.8	214.4
3021.50	5.0	40.0	11.7	93.6
2967.28	2.7	21.6	5.1	40.8
2893.60	0.9	7.2	2.0	16.0
2803.50	2.0	16.0	4.3	34.4
2652.00	3.0	24.0	7.7	61.6
2536.00 2534.80	2.0	16.0	11.2	89.6
2482.70	0.5	4.0	3.0	24.0
2378.30	0.3	2.4	1.0	8.0

portant that the objects should be transparent to visible light so that their cell contents could be examined under the microscope. Several objects were tried but few of them proved satisfactory for the purpose.

To determine their suitability various objects were placed at a distance of 10 cm. from the open water-cooled mercury vapor arc and their reaction to the radiation was observed. The cells of *Chlamydomonas* were found to be very sensitive to the action of the light from the arc. After exposure of 10 minutes their motion ceased, and in about 20 minutes a complete destruction of the cell contents was observed. Due to the motility and small size of the cells it was impossible to confine them within the narrow area (2 mm.²) of irradiation. Although the cells of the filaments of *Spirogyra* were markedly injured by the radiation of the arc after 30-minute exposures, it was difficult to obtain conclusive results, since even non-radiated cells showed a large variation in their response to plasmolyzing agents. *Cladophora* and *Pleurococcus* did not show any marked changes in their cell structure after exposures to the arc for ½ hour. Strips of the epidermis of *Rhoeo discolor* lost their purple pigment after an

exposure to the arc of about 1 hour, but due to their overlapping the vitality of the cells was difficult to judge.

The objects selected as most suitable were leaves of the gametophyte of a common local species of *Mnium* and stamen hairs of *Tradescantia reflexa* Raf. Single leaves of *Mnium* were carefully detached from the stem under the dissecting microscope. Six to nine leaves were then placed in a drop of tap water on a large glass cover-slip 43 × 50 mm. serving as a slide, and covered with a quartz cover-slip. This made it possible to observe the objects with a microscope from both sides. To prevent any pressure on the object, glass capillaries were interposed between the two cover-glasses. The object (i, pl. 42, fig. 2) after a microscopic examination was placed on a vertical adjustable microscope stage (h, pl. 42, figs. 2 and 3) provided with an opening in the center. Three of the leaves were then moved to such a position that the radiation, passing through the slit (2 mm.^{2}) of the monochromator, was focused on them. Thus an area of approximately 0.3 mm.^{2} of each leaf was exposed to the irradiation. The other leaves served as controls.

A similar procedure was adopted for the stamen hairs of *Tradescantia*. They were removed from the filament by means of a pair of pointed scissors, examined to detect possible injury, and then irradiated. At least three hairs were placed in such a position that they were in the path of the light. In each experiment at least eight cells were irradiated.

The exposure of the leaves of the moss plant to the full arc at a distance of 10 cm., for at least 4 hours, produced a decolorization of the chloroplasts. In extreme cases all the chloroplasts were completely deprived of the green color, but transitional stages could be observed. The cell walls appeared as if stained green by the diffused chlorophyll. In the case of one leaf overlapping another, the part shielded had green chloroplasts, although the effect of irradiation was indicated by the green color of the cell walls.

There was no visible change in the starch content of the chloroplast, but in the case of completely decolorized cells no plasmolysis could be produced, indicating destruction of the protoplasmic membrane. A similar effect was obtained by

exposing the leaves for 12 hours to a 1000-Watt Mazda lamp at a distance of 40 cm. However, to produce an effect comparable to that of the arc, an exposure of 12 hours was necessary. After the experiment the irradiated and control leaves were examined. Two of the irradiated leaves were plasmolyzed by an 8 per cent solution of KNO_3, and the third one was placed in a watch-glass with tap water for 3 to 8 hours to observe any possible after-effects of the irradiation. Cells of detached moss leaves used as controls did not show any detectable injury and responded readily to plasmolyzing if left in water for 24 hours.

The *Tradescantia* hairs had purple, vacuolar contents, so that the protoplasmic streaming was readily discernible. Unradiated hairs kept on the stage of the microscope for more than 24 hours showed no visible change in protoplasmic streaming, provided they were handled carefully and not subjected to pressure of the cover-glass. On exposure to the open mercury arc (at a distance of 10 cm.) the streaming ceased in 20 minutes and coagulation of the protoplasm was evident.

All the experiments with monochromatic light on the moss plant were carried out with the object and stage in position B, that is, directly in front of the exit slit of the monochromator (pl. 42, fig. 3). The experiments with the stamen hairs were carried out in position A (pl. 42, fig. 2) for the longer wave lengths and in position B (pl. 42, fig. 3) for the shorter wave lengths. As can be seen from table 1, the intensities at position B were greater than at position A, and were therefore preferred for the moss leaf which was easily centered on the image of the slit by the low power of the microscope. In this position it was not possible to observe the behavior of the cells during irradiation, and the effect was determined by examining the exposed area at the close of the experiments. Position A was preferred for the experiments with stamen hairs, since by interposing a micro-lamp between monochromator and focusing lens (f and j, pl. 42, fig. 2) examinations with 16-mm., 8-mm., and 4-mm. objectives could be made. In the first experiment, with every spectral line tested, microscopic examinations were made at 10- to 15-minute intervals. In the succeeding ones, no examinations were made up to the close of the experiment. In this way a possible

effect of the micro-lamp was eliminated. The adjustment of the hairs over the slit in position B was somewhat difficult because the position of separate cells could not be accurately ascertained. The position of whole hairs, however, was easily determined.

It was of course impossible to test all of the lines of the mercury vapor spectrum or separate the closely adjacent ones. As can be seen from table I, eleven lines (or groups of lines) had been selected. All of them were easily separable with the slits at 0.2 mm. Two to three experiments were conducted for each of the lines used. The time of exposure was in most cases two hours, although in a few instances it was extended to as much as four hours. Longer exposures were not used due to technical limitations.

EXPERIMENTAL RESULTS AND DISCUSSION

The results obtained are represented in tables II and III. No visible distortions in the cell structure were observed with any of the wave lengths or intensities used. All of the irradiated leaves of *Mnium* had a normal appearance. No distortions in the protoplast were visible, and the cells plasmolyzed readily if placed in an 8 per cent solution of KNO_3. No after-effects of the radiation were noticed. It might be possible that a slight discoloration of the chloroplast took place, but if so it was not clearly detectable. In one experiment after an intermittent exposure of eight hours (129.02×10^4 ergs/mm.^{2}) a discoloration comparable to that produced by the open mercury arc was produced. Since this is a single instance and the experiment has not been repeated, it is not taken into consideration in this paper.

In the case of the stamen hairs of *Tradescantia*, protoplasmic streaming was observed in all the cells irradiated. No visible distortion in the cell content was noticed. It seemed that in the case of line 2893 Å the protoplasmic streaming was retarded. The rate of streaming of the protoplasm, however, varied considerably in different cells, hence there is some difficulty in using it as a criterion of vitality.

There is no doubt that stronger intensities, at least for some ultra-violet lines, would produce a killing effect and destruction of the protoplast in plant cells.

From the data available for the energies of ultra-violet monochromatic light required to produce the killing of bacteria and

TABLE II

CORRELATION OF WAVE LENGTH, INTENSITY, TIME OF EXPOSURE, AND INCIDENT LIGHT ENERGY PER SQ. MM., USED IN THE EXPERIMENTS WITH LEAVES OF MNIUM

Wave lengths Å	Incident light intensity ergs/sec/mm.²	Experiment No.	Time of exposure hours	Total incident light energy ergs/mm.² × 10⁴
3654	140.0	I	2	100.80
		II	2	100.80
		III	2	100.80
3341	11.2	I	2	8.06
		II	2	8.06
		III	3	12.10
3131	107.2	I	2	77.18
		II	2	77.18
		III	2	77.18
3021	46.8	I	2	33.70
		II	2	33.70
2967	20.4	I	2	14.69
		II	2	14.69
2893	8.0	I	2	5.76
		II	2	5.76
		III	4	11.52
2803	17.2	I	2	12.38
		II	2	12.38
		III	2	12.38
2652	30.8	I	2	22.18
		II	2	22.18
2536	44.8	I	2	32.26
		II	2	32.26
		III	3	48.38
2482	12.0	I	2	8.64
		II	2	8.64
		III	2	8.64
2378	4.0	I	2	2.88
		II	2	2.88
		III	4	5.76

Constants: Total area irradiated = 2 mm.² Approximate area of each leaf exposed to irradiation = 0.3 mm.² Microscope stage and object in position B (pl. 42, fig. 3).
Effect: No visible changes in cell structure. Normal plasmolysis with 8 per cent KNO_3.

TABLE III

CORRELATION OF WAVE LENGTH, INTENSITY, TIME OF EXPOSURE, AND INCIDENT LIGHT ENERGY PER SQ. MM., USED IN THE EXPERIMENTS WITH STAMEN HAIRS OF TRADESCANTIA

Wave lengths Å	Incident light intensity ergs/sec/mm.²	Experiment No.	Time of exposure hours	Total incident light energy ergs/mm.² × 10⁴
3654	98.0	I*	2	70.56
		II*	2	70.56
		III*	1	35.28
3341	6.0	I*	2	4.32
		II*	2	4.32
3131	42.0	I*	2	30.24
		II*	2	30.24
		III*	2	30.24
3021	20.0	I*	2	14.40
		II*	2	14.40
		III*	2	14.40
2967	10.8	I*	2	7.78
		II*	2	7.78
2893	3.6	I*	1	1.18
	8.0	II	3	8.64
2803	8.0	I*	2	5.76
		II*	2	5.76
		III*	1	2.88
2652	12.0	I*	1	4.32
	30.8	II	2	22.18
2536	8.0	I*	2	5.76
	44.8	II	2	32.26
	44.8	III	3	48.38
2482	12.0	I	1	4.32
		II	2	8.64
2378	4.0	I	2	2.88
		II	3	4.32

Constants: Total area irradiated = 2 mm.² Approximate number of cells irradiated = 8.

Effect: No visible injury to the cell. Protoplasmic streaming continued.

In experiments indicated by asterisk (*) microscope and object were in position A (pl. 42, fig. 2); in the others, in position B (pl. 42, fig. 3).

paramecia (Gates, '29, Weinstein, '30), it was anticipated that the comparatively strong energies used in the present investigation would be sufficient to produce a similar effect on plant cells. This is apparently not the case.

TABLE IV

COMPARISON OF TOTAL ENERGIES (EXPOSURE TIME × INTENSITY) USED IN THIS INVESTIGATION WITH THOSE USED BY GATES AND WEINSTEIN

Wave length	Ergs/mm.²			
	Used in present investigation		Gates*	Weinstein
Å	*Tradescantia*	*Mnium*	necessary to kill 100% of bacteria	necessary to kill paramecia
3654	705.600	1,008,000		
3341	43,200	80,600		
3131	302,400	771,800		19,629
3021	144,000	337,000	13,000	10,850
2967	77,800	146,900	3,000	
2893	25,900	57,600	725	
2803	57,600	123,800	475	2,473
2652	86,400	221,800	350	2,162
2536	322,600	322,600	325	2,284
2482	86,400	86,400	350	
2378	28,800	28,800	540	

* Calculated from curves Gates ('29), p. 240.

TABLE V

COMPARISON OF INTENSITIES (ENERGY PER SECOND) USED IN THIS INVESTIGATION WITH THOSE USED BY GATES AND WEINSTEIN

Wave length	Ergs/mm.²/sec.			
	Intensities used in this investigation		Intensities used by Gates	Intensities used by Weinstein
Å	*Tradescantia*	*Mnium*	Bacteria	Paramecia
3654	98.0	140.0		
3341	6.0	11.0		
3131	42.0	107.2		7.27
3021	20.0	46.8		5.48
2967	10.8	20.4		
2893	8.0	8.0		
2803	8.0	17.2		1.33
2652	30.8	30.8	11.0	2.72
2536	44.8	44.8		1.12
2482	12.0	12.0		
2378	4.0	4.0		

If we compare the total energies (time of exposure × incident intensity) of this investigation with those used by the two

authors (table IV), it is apparent that much larger energies were used in this experiment.

Although the Roscoe-Bunsen photochemical reciprocity law has been shown to be fairly accurate for some biological objects, there still might be some doubt as to its applicability for exposures as long as two hours. Allowance in this case must be made for a possible concurrent recovery process of the organism during irradiation. However, table V shows that the intensities used in this experiment were larger by a factor of 3 to 40, and therefore the greater energies were due to increase both of intensity and time of exposure. It is therefore evident that the plant cells used are much more resistant to the lethal action of ultra-violet than bacteria and paramecia.

Several attempts have been made to explain the lethal action of ultra-violet radiation. Its killing action is usually ascribed to the destruction or precipitation of some of the constituents of the protoplasm. Henri ('12) found that the abiotic power of ultra-violet rays is proportional to the coefficient of absorption of the protoplasm. Gates ('28) believes that the destruction of certain nucleoproteins is responsible for the killing of the cell. However, it is clear that the resistance of a cell to the action of the rays will depend also on its structural characteristics, such as size of the cell, nature of its cell wall, presence or absence of pigments, etc. Henri ('12) showed that in small cells the entire protoplasm was affected by the rays, whereas in larger cells only a surface reaction resulted. Schulze ('09) noticed that the cell wall and especially the middle lamella are the parts of the cell which absorb most of the radiation if exposed to line 2800 Å. The comparatively high resistance of our objects to the lethal action of ultra-violet radiation might then probably be explained by the characteristic structure of the cells of higher plants.

SUMMARY

1. The necessity of quantitative data for the study of the effect of ultra-violet light on plants is discussed.

2. An experimental arrangement for securing monochromatic light of measured intensities and its application for use with plant objects are described.

3. Leaves of *Mnium* and stamen hairs of *Tradescantia* are exposed to the radiation of eleven lines of the mercury spectrum.

4. It has been shown that no visible injury to the cells resulted from exposure to relatively strong intensities.

5. By comparing these results with those obtained by other workers it has been shown that the plant cells used are very much more resistant to the lethal action of ultra-violet light than bacteria and paramecia.

Acknowledgments

I wish to express my deep appreciation to Dr. E. S. Reynolds, under whose direction the investigation was carried out, for his assistance and suggestions during the progress of the work and preparation of this paper. I am also indebted to Dr. George T. Moore for the use of the facilities and library of the Missouri Botanical Garden, and to Dr. Lester C. Van Atta for assistance and suggestions in setting up the apparatus. The monochromator used in this investigation was loaned through the courtesy of the Bausch and Lomb Optical Company and the Committee on the Effects of Radiation upon Living Organisms of the Division of Biology and Agriculture, National Research Council. The Burdick water-cooled mercury arc was kindly placed at my disposal by the Dick X-Ray Company of St. Louis, Mo.

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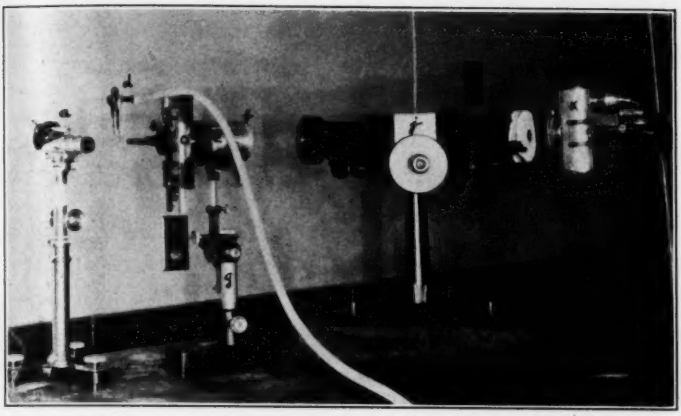
Explanation of Plate

PLATE 42

Fig. 1. Position of monochromator, focusing device, and thermopile during intensity measurement (A).

Fig. 2. Position of monochromator, focusing device, and stage supporting object during irradiation (A).

Fig. 3. Microscopic stage and object in position B close to exit slide of monochromator.



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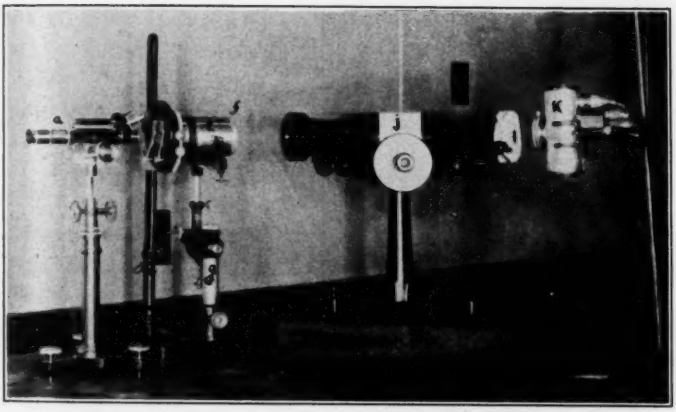

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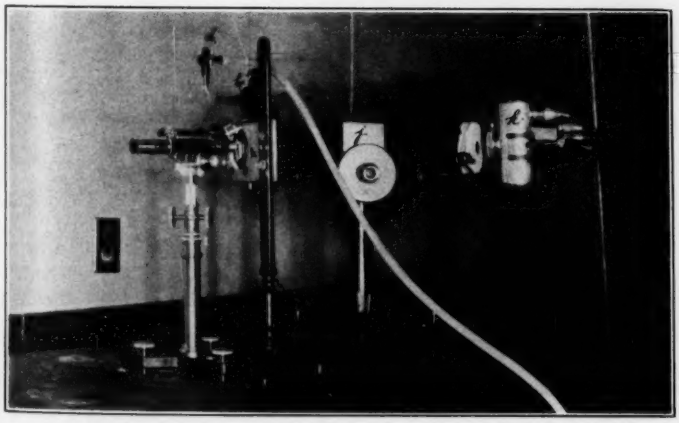

3

BUCHOLTZ—MONOCHROMATIC ULTRA-VIOLET LIGHT

THE CYTOLOGY OF FUNARIA FLAVICANS MICHX. WITH SPECIAL REFERENCE TO FERTILIZATION[1]

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I. Introduction and History

The desirability of a study of fertilization in the Mosses was suggested by the fact that this is the one great group of plants in which no thorough investigation had been undertaken concerning nuclear fusion. The question of fertilization in the Liverworts, which are similar to the Mosses in their structural aspects, has received considerable attention in recent years. In the Liverworts three distinct types of nuclear fusion are encountered. Inasmuch as the observations regarding fertilization in the Mosses are so meagre and since the processes in the Liverworts are so diverse, the present investigation was undertaken.

The literature contains fragmentary observations on the subject, the earliest of which is that of Hofmeister ('62), who observed in *Funaria* an antherozoid moving down the neck of an archegonium which was ready for insemination. In the case of dioecious species no fruit or sporophyte was formed unless male and female plants were growing in the same locality. He observed that the young sporophyte when consisting of from one to four cells remained free in the ventral cavity, but, after further division, grew down into the tissue of the archegonium.

Roze ('72), studying the development of the archegonium in *Sphagnum*, depicted an archegonium with several antherozoids in the neck canal and one antherozoid in contact with the egg. The antherozoids entered with the ciliated portion foremost and remained in the ventral cavity. The thickness of the archegonial wall prevented him from determining the progress of penetration of the antherozoid into the egg.

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

Arnell ('75) observed, during an examination of archegonial material of *Discelium nudum* (Dicks.) Brid., one plant of which the archegonium had a visible canal. During his observations the top cells separated and the archegonium opened. The antherozoids were drawn to the mouth of the archegonium as if attracted by a magnetic power. In addition he noticed a rocking motion of the egg due to the movement of the antherozoids which were surrounding it.

Gayet ('96), in connection with his study of archegonial development in *Bryum capillare* L., observed antherozoids swimming down the neck canal and one of them penetrating the egg. After penetration the antherozoid assumed a crescent shape and became located above the egg nucleus.

In *Fissidens incurvus* Schwaegr., Gayet perceived that in fertilization a large number of antherozoids penetrated the egg, but that only one united with the egg nucleus. The antherozoid, in the cytoplasm of the egg, became crescent-shaped at first and then spherical. The female nucleus possessed four chromosomes which were attracted to the male nucleus. After fusion only four chromosomes were discernible in the fertilized egg, and Gayet inferred that each male chromosome fused with a female.

W. and J. Van Leeuwen-Reijnvaan ('08a) reported a very bizarre process in the fertilization of *Polytrichum*. From their observations they inferred that reduction divisions occurred both in the archegonium and in the antheridium. The ventral canal cell and the egg then fused, and the product of this fusion was fertilized by two antherozoids. In this way the sporophyte had double the original number of chromosomes. The same authors reported a similar situation in *Mnium*.

Wilson ('09) found in *Mnium*, however, that reduction division took place in the division of the spore-mother-cells to form spores. In studying spermatogenesis in other Bryophytes he found no evidence of a reduction division in the formation of the antherozoids.

Vandendries ('12) studied spermatogenesis in *Polytrichum* with reference to the chromosome number. He showed quite conclusively that there was no double reduction occurring in the formation of the antherozoids.

Walker ('13) found no fusion of the egg with the large ventral canal cell of *Polytrichum*. He contended that the bizarre process described by J. and W. Van Leeuwen-Reijnvaan was doubtful since his studies showed no such process occurring.

Bryan ('20) described in complete detail the fusion of the ventral canal cell and the egg of *Sphagnum subsecundum* (Nees) Limpr. His results indicated that the number of cases in which this abnormal fusion occurred about equalled those in which the ventral canal cell had disintegrated. He reported two cases in which the egg nucleus disintegrated and in which the nucleus of the ventral canal cell remained distinct and sharply defined.

Harvey-Gibson and Miller-Brown ('27) published a preliminary note on the fertilization of Bryophytes. Their work consisted of observations made on mites which visited both male and female heads of *Polytrichum commune* L. The mites carried sperms on their bodies from antheridial heads to archegonial heads and in this way brought about insemination of distant archegonial heads.

The Liverworts, on the other hand, have received considerable attention within recent years. Rickett ('23) has summarized the earlier literature on the subject, and it need not be repeated here.

In *Sphaerocarpos*, according to Rickett, the male nucleus after penetration into the cytoplasm of the egg swells markedly, becoming spherical and reticulate but remaining smaller than the female nucleus. The two gametic nuclei come into contact but remain distinct until each has organized its chromosomes preparatory to mitosis. The two nuclear membranes then disappear and a spindle figure is formed, thus initiating the metaphase of the first embryonic division.

Showalter ('26, 27a, 27b, '28) has made careful studies of the fertilization processes in three of the Anacrogynae. In *Riccardia* ('26) the male nucleus remains, with scarcely any perceptible change of form, in the cytoplasm of the egg for from twenty-nine to thirty-six hours. Then it penetrates endwise and passes slowly into the female nucleus, where it forms first a vesicle of deeply staining chromatic material, and later a compact reticulum that loosens up more and more until after three to four days it

is no longer distinguishable from the reticulum of maternal chromatin.

In *Pellia* ('27b) the male nucleus, after penetration into the egg, moves slowly toward the female nucleus and gradually assumes a reticulate form. The cytoplasm between these two nuclei recedes, leaving the mass of paternal chromatin almost in contact with the membrane of the female nucleus. The membrane of the female nucleus seems to dissolve in the region of contact with the paternal chromatin, and a common membrane encloses the paternal chromatin and the female nucleus. The union of the two nuclei occurs usually the second day after insemination. The paternal chromatin quickly assumes the condition of the maternal chromatin, and except for the presence of two nucleoli the dual nature of the fusion nucleus is distinctly evident for a short time only.

In *Fossombronia* ('27a) actual penetration of the male nucleus into the female nucleus was not observed, although Showalter found that after forty-eight hours the chromatic mass about the nucleolus had become a reticulum which occupied approximately one hemisphere of the nuclear cavity. In the other hemisphere was a dense mass of chromatic substance which was more intensely stained than the chromatic reticulum, and it seemed probable that this dense mass was the substance of the male nucleus.

Showalter ('28) has studied hybrid fertilization in four varieties of *Riccardia pinguis* (L.) S. F. Gray. In this study he found that nuclear fusion between the four types was in accord with that described in his earlier paper on *Riccardia* ('26).

The salient points in these investigations are shown in table II, where they are compared with the results obtained in *Funaria*.

II. Materials and Methods

Funaria flavicans Michx. is similar in its morphological features to *Funaria hygrometrica* (L.) Sibth., with which it is often associated in nature. It may be distinguished from *Funaria hygrometrica* by its smaller size, its erect pedicel, and its more pointed leaves. The capsule, which is furrowed less deeply than that of *Funaria hygrometrica* and which has a non-apiculate lid,

matures a week or two earlier. *Funaria hygrometrica* is almost cosmopolitan in its distribution, whereas *Funaria flavicans* has been reported only from the mid-central and southern portions of the United States. Both species are monoecious. *Funaria flavicans* is abundant around St. Louis, Missouri, and was found to grow well in cultures. Hence, it was selected for the experimental work described in this thesis.

Mature sporophytes were obtained in the spring of 1929, near Festus, Missouri, where a great number of plants were growing on an outcrop of St. Peter sandstone. Specimens of the material were sent to Dr. A. J. Grout, who kindly verified the identification. The spores were sown on a sterile mixture of sand and soil in six-inch pots which were then set in granite pans containing tap-water. This procedure permitted the soil to remain moist and at the same time prevented spontaneous insemination. The cultures were kept in a north greenhouse in which the temperature was relatively cool. When both archegonia and antheridia were mature insemination was brought about by flooding the cultures. The pots were tightly corked from below, placed in a container of water, and then covered with water. These precautions were taken in order that the water would not seep down into the soil, carrying the antherozoids with it. At the end of half an hour, that being the time determined necessary for the antherozoid to escape from the gelatinous envelope surrounding it, the pots were removed from the container, uncorked, and the excess water permitted to drain out at the bottom of the pots. Fixations were made at intervals after flooding. The killing fluids employed were chromo-acetic, Flemming's medium, Showalter's modification of Flemming's medium ('26), Navaschin's chromo-acetic formalin as described by Babcock and Clausen ('29), and Benda's fluid. Although Flemming's medium and Benda's fluid produced perceptible plasmolysis, Showalter's modification and Navaschin's fixative gave excellent results.

After washing, dehydration, infiltration and imbedding in paraffin, the material was sectioned and stained. Sections cut 12 μ in thickness often included the entire egg in one section. The material was stained, some with Flemming's triple stain,

some with Haidenhain's iron-alum haematoxylin, and some with the gentian violet-iodine combination. The iron-alum haematoxylin did not give as clear nuclear differentiation as was obtained with the triple or with the gentian violet-iodine combination.

In studying the slides, recourse was made to the standardized scheme of systematic and objective observation developed by Fry ('30) in connection with his studies of fertilized echinoderm eggs and recently outlined by him.

III. Observations

1. Spore germination and development of the leafy shoot

The first visible indication of germination of spores sown on soil is the modification in spore color. The spores when sown have a definite orange color, but with the development of more chlorophyll in the protoplasts the orange color is gradually transformed first to a brown, then to a deeper brown, until the distinction between the brown color of the spore and the green color of the developing protonemata is scarcely perceptible. These faint patches of green color are discernible three days after the spores are sown. The development of the protonemata continues rather rapidly, and under favorable temperature conditions, around 20° C., the entire pot is covered with protonemata within four weeks. After six to seven weeks the first leaves of the gametophores appear. Their development continues, and within three months the antheridia are discernible at the tips of leafy branches as small green knob-like structures. As the antheridia mature they change in color from green to bright orange, and with the discharge of the antherozoids they become very dark brown.

Since *Funaria flavicans* Michx. is monoecious the archegonia are developed on the same plant as are the antheridia. However, the archegonial heads arise as lateral branches of the male gametophore. Their development takes place at a later date, and the relationship of the archegonial branch to the antheridial when the antheridia are maturing is indicated in fig. 1. Following insemination and fertilization the archegonial branch develops rapidly and quickly surpasses the antheridial branch in size, until the latter is quite insignificant in comparison with the former.

The young sporophyte is visible to the unaided eye within ten days after fertilization. At this time its structure is exceedingly long and narrow, indicating a very small mass of potential sporogenous tissue, but this tissue increases in amount with

Fig. 1. Relationship of archegonial branch (right) to the antheridial branch when antheridia are maturing.

continued growth of the sporophyte. The sporophyte with fully developed spores reaches maturity five weeks after insemination.

2. DEVELOPMENT OF THE ANTHERIDIUM

The antheridium develops from a superficial cell (pl. 43, fig. 1). Nuclear division takes place and a cross wall is laid down between the two daughter nuclei (pl. 43, fig. 2). The division of these cells continues, with walls being laid down at various angles, until a many-celled structure is developed (pl. 43, fig. 3). The outer layer of cells develops into the wall of the antheridium, and the two cells at the apex are characterized by their unusual size (pl. 43, fig. 4). These two cells function in the discharge of

the antherozoids. The antherozoid when discharged is surrounded by a gelatinous envelope which is readily dissolved, after which the antherozoid may be observed swimming about (pl. 43, figs. 5–6). In material which has been fixed and stained the two cilia are very distinct, but in living material these structures are not readily observed.

In the antheridial head are found sterile hairs or paraphyses which are multicellular and contain numerous chloroplasts. In very young heads it is observed that these paraphyses are filiform and very similar to those found in the archegonial heads (pl. 43, fig. 7). The changes undergone in the transition from the filiform to the clavate condition are unusually interesting and apparently have never been described (pl. 43, figs. 8–9). The nucleus in the one-celled filament possesses a very large nucleolus which in a later stage is apparently in the process of division and represents what might be interpreted as an intra-nuclear division (pl. 43, fig. 10), such as is found in certain of the lower fungi. The cytoplasm of the young paraphysis contains very definite rod-shaped structures that increase in thickness and later are distinguished as early stages in plastid development. In comparing living material with fixed material the stages in plastid development are readily observed. In the unicellular paraphysis the chloroplasts are elongated structures which appear as rods in fixed material. These chloroplasts change from the elongated rods into small spherical bodies. In the mature clavate paraphysis the chloroplasts have increased in size and their structure is characteristic of the mature plastid.

There is a considerable number of mature antheridia in one head with the majority in the same stage of development, that is, nearly all the antheridia develop concurrently and mature almost simultaneously. Occasionally an antheridial head is found in which a number of developmental stages was found, but this was somewhat rare.

3. DEVELOPMENT OF THE ARCHEGONIUM

The archegonium, like the antheridium, develops from a superficial cell, and in the very early stages it is impossible to distinguish a young archegonium from a young antheridium

(pl. 43, fig. 11). In somewhat later stages the paraphyses may be used as a criterion, since they are characteristically different in the two kinds of heads. In the archegonial head they are always unicellular and filiform and may be distinguished from the young filiform paraphyses of the antheridial head by the less dense cytoplasm and by the fact that they are long and slender, whereas those of the antheridial head are shorter and more nearly uniform in transverse diameter (pl. 43, fig. 14).

The cell which gives rise to the egg and the ventral canal cell is located in the basal portion of the archegonium and completely fills the ventral cavity (pl. 43, fig. 15). The division of this cell is somewhat unequal, the egg receiving approximately three-fourths of the cytoplasm of the original cell, the ventral canal cell the remaining fourth. The fact that there is an invagination of cytoplasmic material at the point where the two cells divide gives one the impression that division is brought about by furrowing rather than by formation of a cell plate, but this invagination may be due to the rounding up of the newly formed cells (pl. 43, fig. 16). An insufficient number of observations prevents the writer from making any precise deductions.

The ventral canal cell, after formation, has a clearly defined nucleus. This disintegrates eventually and in later stages is recognized merely as a dense mass within the cytoplasm (pl. 44, fig. 17). When this nucleus ceases to be recognizable as such the beginning of disorganization of the neck canal cells may be observed, the ventral canal cell disintegrates along with the other canal cells, and the egg is ready for fertilization.

The number of archegonia in a head varies from one to many. These are generally at various stages of development, although there may be two mature archegonia in one head at the same time. Both of these archegonia may contain eggs in the same stage of fertilization. This is encountered in about 17 per cent of the material examined, and in one plant three mature archegonia were found with their eggs in precisely the same stage of fertilization. The archegonia apparently exercise no ill effects on each other, for several instances were noted in which two young sporophytes in a multicellular condition were found in the same head. These sporophytes showed no indication of being in a dwarfed condition.

4. FERTILIZATION AND NUCLEAR FUSION

In material fixed immediately after flooding, the spherical egg with densely granular cytoplasm is found either in the central portion of the ventral cavity or toward the bottom. The unfertilized eggs range in diameter from 7.0 to 8.6 μ, whereas the ventral cavities range from 8.0 to 12.6 μ. The nucleus, which occupies the central portion of the egg, is from 2.7 to 3.3 μ in diameter and possesses a very large nucleolus. Very little chromatin material, other than the nucleolus, could be definitely recognized. However, a distinct granular zone was differentiated about the nucleolus (pl. 43, fig. 18). At this particular stage only one archegonium was found in which the antherozoids had passed down the canal and were near the egg. The gelatinous envelope which surrounds the antherozoid when discharged from the antheridium requires from twenty to thirty minutes for dissolution. Inasmuch as this period of time is required and since the neck canal is exceedingly long, it is probable that in material fixed immediately after flooding the antherozoids have not had the opportunity to reach the egg in the archegonium.

With complete dissolution of the gelatinous envelope the antherozoids swim down the neck canal and into the venter. A large number of them approach the egg so that it has the appearance of being covered with very fine threads (pl. 43, fig. 19). Immediately following the entrance of the antherozoids into the venter a mucilaginous plug appears in the canal (pl. 43, fig. 20). This plug seems to be a secretion of the first two tiers of cells above the venter, inasmuch as it is always found associated with these two tiers of cells and never with any others. The plug, furthermore, is connected in some way with the process of fertilization. In all archegonia in which antherozoids have entered, the plug is present and remains not only throughout the fertilization process but also in early stages of sporophytic development. A few archegonia are found in which the ventral canal cell has not completely disintegrated and into the venter of which antherozoids have entered. In these no indication of the mucilaginous plug is discernible. The plug, which stains less deeply than the egg or any of its components, appears to have the ability to prevent the entrance of any more antherozoids, because

in the majority of cases there is present above the plug a large number of antherozoids tangled together. In some instances one to several antherozoids have been caught in this mucilaginous secretion.

The antherozoids approach the egg from all angles and tend to become closely adpressed to its surface which is slightly indented along the lines of contact (pl. 44, figs. 21–22). Those antherozoids, which become adherent to the egg, take a deeper stain than do those which are present in the ventral cavity and in the canal, since whenever the triple stain has been used the former are violet in color whereas the latter are red—depending upon the intensity of the stain. Notwithstanding the fact that the antherozoids lose their cilia on becoming adpressed to the egg, those present both in the canal and in the cavity are still equipped with cilia.

The antherozoid is a long slender structure with the anterior region somewhat enlarged and spherical. The two cilia which are present are attached to the antherozoid at this anterior region.

The antherozoid after becoming attached to the surface of the egg begins to pierce the membrane in its spherical portion (pl. 44, fig. 23). The substance of the antherozoid gradually passes into the cytoplasm of the egg, after which the original form is resumed. The antherozoid, in 63 per cent of the cases observed, penetrates the surface of the egg in the side toward the base of the archegonium. If penetration has not occurred in this region it takes place at one side (30 per cent of all cases observed) of the nucleus and only rarely above (7 per cent) the level of the nucleus. The time required for penetration is rather short, material fixed two hours and twenty minutes after insemination showing antherozoids in the cytoplasm of all the eggs in condition to be fertilized.

The entrance of an antherozoid into the cytoplasm of the egg seems to stimulate it to increase gradually in size. With this gradual increase in size there is a concurrent increase in the size of the ventral cavity as well as in the width of the archegonium. The size of the egg nucleus, however, is affected only slightly by this penetration of the antherozoid. Table 1 gives the average

measurements of these structures and indicates the gradual increases in size.

TABLE I

AVERAGE MEASUREMENTS

Fixation number	Interval after flooding	Egg diameter in microns	Ventral cavity diameter in microns	Archegonium diameter in microns
1	5 minutes	7.7	10.1	——
2	35 minutes	7.1	10.9	——
3	1 hr. 35 min.	8.5	12.3	——
4	2 hr. 20 min.	8.3	12.3	37.9
5	3 hr. 30 min.	8.5	12.8	35.6
6	4 hr. 30 min.	8.7	13.8	40.1
7	5 hr. 30 min.	8.8	13.8	40.3
8	6 hr. 30 min.	8.6	13.0	37.0
9	7 hr. 30 min.	9.3	12.6	40.0
10	8 hr. 30 min.	8.3	14.0	33.8
11	9 hr. 30 min.	8.9	12.6	38.6
12	10 hr. 30 min.	9.0	12.5	36.4
13	11 hr. 30 min.	8.9	13.9	39.2
14	14 hr.	9.1	12.3	42.6
15	16 hr.	8.8	14.1	39.9
16	18 hr.	8.9	13.1	44.7
17	18 hr. 30 min.	9.2	15.3	39.2
18	22 hr. 20 min.	9.5	13.0	41.3
19	23 hr. 30 min.	9.6	14.0	40.9
20	28 hr. 10 min.	9.3	13.7	38.1
21	45 hr. 20 min.	9.9	14.2	46.6
22	45 hr. 55 min.	9.9	15.5	45.9
23	48 hr. 45 min.	10.0	16.6	46.3
24	93 hr.	10.2	16.8	47.1

The variations in the widths of the ventral cavity and of the archegonium are explained by the fact that the region containing the egg is not always the median portion of the archegonium. If the egg is situated toward the outer area of the ventral cavity the archegonium will be narrower in width at this point than in the region directly at the center of the ventral cavity.

Penetration, moreover, is not restricted to a single antherozoid. Instances of polyspermy, however, are relatively few, only a small number being noted and these in the very early stages soon after

insemination. It is quite likely that the supernumerary antherozoids disintegrate, inasmuch as no case was found at a later stage in which they were present in the cytoplasm of the egg. Plate 44, fig. 26, shows an egg in which the spherical portions of several antherozoids have penetrated, the elongated portions not being readily distinguishable in the dense cytoplasm.

In two instances where the ventral canal cell had not completely disintegrated both the aforementioned cell and the egg were surrounded by antherozoids. There is no indication, however, that the antherozoids ever penetrate the ventral canal cell.

The antherozoid does not remain in the cytoplasm in a quiescent condition. It becomes shorter and thicker and passes to a position near the female nucleus immediately after penetration is completed (pl. 44, fig. 27). In material fixed three hours and thirty minutes after insemination the male nucleus has come in contact with the egg nucleus (pl. 44, fig. 28).

There was but one case observed in which the supernumerary antherozoids were still surrounding the fertilized egg. Those which do not penetrate the membrane apparently disintegrate, for with this one exception no instances have been found in which the supernumerary antherozoids remain adjacent to the egg longer than three hours and thirty minutes after insemination. On the other hand, those antherozoids which are present in the cavity are still recognizable as such in material fixed ten hours and thirty minutes after insemination.

The cytoplasm becomes less dense with the entrance of the antherozoid, and material fixed in Flemming's medium and stained with the iron-alum haematoxylin shows the presence of very definite rod-like and spherical bodies (pl. 44, fig. 35). The dense mass about the nucleolus becomes less granular and moves toward the periphery of the nuclear cavity, leaving a clear zone about the latter structure. The cells of the archegonium become vacuolated, and in the basal portions mitotic figures occasionally are found.

The male nucleus as it comes in contact with the female nucleus causes a depression in the surface of the latter. The antherozoid or sperm nucleus becomes shorter and thicker and is distinguished at one side of the female nucleus or below it as

a slightly elongated ovoid structure (pl. 44, figs. 29, 30). The male nucleus penetrates the female nucleus, and its chromatin substance passes into the female nuclear cavity where it assumes a more or less definitely ovoid form (pl. 44, figs. 31, 33, 34). The exact method of penetration could not be determined because of the minute size of the nucleus. Whether the membrane disappears at the point of contact has not been definitely determined. The membrane is not visible at the point where the male nucleus is in contact and later enters, but immediately after complete entrance a membrane is again very distinct and definite.

The male nucleus, moreover, does not remain in a resting state. The nucleolus of the egg nucleus, which is in reality the condensed reticulum enclosing the true nucleolus, tends to become vacuolate after entrance of the male nucleus into the female nucleus, and the mass of paternal chromatin is attracted to it (pl. 44, fig. 32). The dense mass which previously surrounded the condensed reticulum has practically disappeared, and about the two masses present in the nuclear cavity there is distinguished a zone which is quite clear. During the entrance of the paternal chromatin a definite staining area is evidenced, indicating the penetrating substance. The two distinct masses of chromatin tend to come together in the center of the cavity. The nuclear membrane becomes irregular in outline and eventually disappears completely (pl. 44, figs. 36–39). The two chromatin masses come into contact and gradually fuse, the two bodies being vaguely distinct and discernible only as darker-staining regions (pl. 44, fig. 40; pl. 45, fig. 41). The complete intermingling of the two masses of chromatin is more or less gradual.

The fused mass of chromatin is easily recognized by the fact that it is a somewhat spherical body with specific regions that are much darker than others. It remains in the central portion of the egg and without any perceptible membrane for several hours after fusion. During this time there is no apparent change in the structure of the fusion product. The cytoplasm is less granular at this stage and tends to become somewhat vacuolated toward the periphery of the egg. After ten to twelve hours the granular portion of the cytoplasm tends to become aggregated about this fusion nucleus. The aggregated cytoplasm

shows a tendency to become dense and gives the appearance of a definite granular zone similar to the one observed in the earlier stages. As this zone increases in density the outlines of a membrane being laid down become visible, at first considerably irregular, but gradually more regular and definite (pl. 45, figs. 44–48).

The fusion nucleus, as it is now recognized, remains in a resting stage for some time. A fertilized egg in this stage is distinguished from an egg just prior to insemination by its larger size, as well as by the appearance of the condensed reticulum.

In some of the material fixed forty-five hours and twenty-five minutes after insemination, the mucilaginous plug indicating that insemination has occurred, very definite plastid-like bodies are observed in the egg. These bodies are much too large and regular in appearance to be considered as chondriosomes. The condition of the nucleus is somewhat masked from view by the presence of these plastid-like bodies. In later stages, however, no such bodies are discernible, and it is a matter of speculation whether their preservation in this case is attributable to the particular fixative used, Benda's fluid, or whether the egg was not fertilized, as a result of which the plastid-like bodies developed.

The nucleus remains in the resting condition for a considerable period, after which it undergoes the changes for the prophase of the first division. The condensed reticulum, or the body which represents the fusion of the maternal and paternal chromatin, presents an appearance similar to that found in other nuclei at an early prophase stage in the division process. The chromatin becomes transformed into a spireme which is located not in the peripheral portion of the nuclear cavity as is customary, but within the region previously occupied by the condensed chromatin (pl. 45, fig. 50).

The first division of the nucleus and cell is transverse to the long axis of the egg and the archegonium. The two daughter nuclei which are formed tend to pass through a short resting stage before going through the second division which is at right angles to the first. There is an enormous increase in the size of the cell with the formation of the daughter nuclei. An embryo

in the bi-nucleate condition has a diameter of 25 μ, whereas the fertilized egg in the later stages before division has a diameter of 11 μ. The nuclei are quite large, possessing large nucleoli, and present an appearance similar to that found in the resting nuclei of the mature egg (pl. 45, figs. 51, 52). Further divisions occur rapidly until a multicellular sporophyte is formed. This sporophyte remains free in the ventral cavity and does not grow downward into the tissue of the archegonium until some time later. The archegonium increases considerably in size, and this increase is correlated with the increase in size of the young sporophyte.

5. SPORE FORMATION

The sporogenous tissue originates as a single row of cells toward the outer periphery of the columella. The cells are at first rectangular and are in an active stage of division. They increase in size and become rounded off so that at the time they are matured into spore-mother-cells they are quite spherical in shape.

The nucleus of the spore-mother-cell divides, and the resulting daughter nuclei go to opposite ends of the cell. These daughter nuclei do not appear to undergo a resting stage but pass from a very late telophase into the early prophase of the second division. In the second division the plane of division of one daughter nucleus is at right angles to that of the other daughter nucleus. This conclusion is reached from the fact that the majority of spore-mother-cells shows only three nuclei in focus, the fourth nucleus being seen when the focus is changed (pl. 45, figs. 54–56).

Cytokinesis of the spore-mother-cells is by cell-plate formation, the cytoplasm displaying no indication of furrowing either after the first division or after the second division. No walls are laid down after the first division, but those formed after the homeotypic division are laid down before the daughter nuclei are completely reconstructed.

6. CHROMOSOMES

An attempt was made to determine not only the structure of the chromosomes but also the specific number. Mitotic figures are frequently found in various tissues of the plant. The lower

portion of the archegonium shows a large number of mitotic figures, one of which gives an excellent polar view (pl. 45, fig. 57). By careful focusing, ten chromosomes can be brought into view. It is very likely that these represent both poles, inasmuch as the sections were cut rather thick. With the highest magnification available, it has been impossible to determine the exact number of chromosomes. Mitotic figures in the antheridium are less helpful than those of the archegonium. Certain of the spore-mother-cells, after the first division, show the chromosomes being transformed into the spireme of the daughter nuclei. Such figures display approximately ten short rods becoming more or less entangled with one another. Previous to the homeotypic division very definite chromosomes, unusually small and irregular in shape, were observed, but the exact number could not be determined.

IV. Discussion

The fertilization process in *Funaria flavicans* varies considerably from the processes described in the Bryophytes which have been investigated by other authors.

The mature egg of *Funaria*, at the time of insemination, is much smaller than that of any Liverwort studied. In *Sphaerocarpos* the egg is 40 x 20 μ, in *Fossombronia* it is about 25 μ in diameter, in *Riccardia* about 20 μ, whereas in *Pellia* no actual measurements are given although it is stated to be larger than that of *Riccardia*. In *Funaria* the average size of the egg at the time of insemination is 7.7 μ in diameter, a third the size of any other egg studied. The nucleus is correspondingly small. In *Sphaerocarpos* the nucleus of the mature egg is 13 x 10 μ, in *Fossombronia*, as well as *Riccardia*, it is about 10 μ, and in *Funaria* 2.9 μ, a third the size of the other nuclei. The volumes, determined from these measurements, give a much better indication of the comparative sizes of the structures studied in these different forms. These volumes are as follows:

	Egg	Nucleus
Sphaerocarpos	14,137.9 cu. μ	796.3 cu. μ
Fossombronia	8,181.9 cu. μ	523.6 cu. μ
Riccardia	4,189.0 cu. μ	523.6 cu. μ
Funaria	239.1 cu. μ	12.8 cu. μ

The relatively small size, not only of the egg but of the nucleus as well, increases the technical difficulties and may be one of the reasons that no previous cytological work has been done on the fertilization of the Mosses.

Rickett ('23) observed a quantity of mucilaginous material resulting, supposedly, from the disintegration of the ventral canal cells and the neck canal cells in *Sphaerocarpos*. This material not only filled the neck canal but also a part of the venter and was seen extruding from the neck. No such mucilaginous material was observed in the other Liverworts that have been studied, nor was it seen in *Funaria*, although an apparently similar phenomenon was observed. This will be discussed later.

The manner of the penetration of the antherozoid into the cytoplasm of the egg is dissimilar in the Bryophytes that have been studied. In *Sphaerocarpos* the membrane of the egg is very delicate and thin, and actual penetration of the antherozoid into the cytoplasm of the egg was not observed. The process is presumably instantaneous, for in eggs fixed fifteen, twenty, or forty-five minutes after insemination the antherozoid was observed as a slender curved body in the cytoplasm. The entrance of the antherozoid was not restricted to any one portion of surface of the egg. Some entered at the distal end, others at the basal end, whereas still others entered at one side.

In *Riccardia* penetration of the antherozoid is a gradual process. The antherozoid becomes applied to the surface of the egg which becomes depressed along the line of contact, and the antherozoid or its nucleus passes laterally into the cytoplasm. Material fixed twenty to thirty minutes after insemination showed the antherozoid in the surface membrane of the egg. In *Pellia* penetration is similar to that in *Riccardia*. In *Fossombronia* penetration seems to be nearly instantaneous, accompanied by a swelling of the antherozoid. In plants fixed six minutes after insemination a number of eggs was found which had been penetrated by antherozoids.

In *Funaria* the time required for penetration is somewhat longer. In material fixed one hour and thirty-five minutes after flooding only three out of seventeen eggs examined showed

partial penetration of antherozoids. However, in material killed two hours and twenty minutes after flooding all eggs showed complete penetration of the antherozoid. In *Funaria*, moreover, there is a tendency for the antherozoid to enter at the basal end of the egg, although some showed penetration at one side and a very few at the distal end of the egg. It is doubtful whether this basal end functions as a "receptive spot" as stated by Shaw ('98) in *Onoclea*. In *Onoclea* there is a definite concavity of the egg, which is not present in *Funaria*.

The length of time which the antherozoid remains in the cytoplasm is not the same for all Bryophytes that have been studied. In *Sphaerocarpos* the male nucleus remains in the cytoplasm approximately forty-six hours; in *Pellia* for about twenty-four to thirty-six hours, during which it undergoes a change in form, preparatory to nuclear fusion; in *Riccardia* it remains almost without change of form in the cytoplasm of the egg for the same length of time, after which it begins an endwise penetration of the female nucleus. In *Fossombronia* actual penetration of the male nucleus into the female nucleus was not observed. In *Funaria* the male nucleus does not remain in the cytoplasm for a long period of time, but almost immediately undergoes a change in form and position. In eggs fixed three hours and thirty minutes after flooding the male nucleus, in the majority of cases, was found to be in direct contact with the nucleus of the egg.

The greatest variation between these species exists in the methods of nuclear fusion. *Sphaerocarpos* displays the type in which the nuclei come in contact with each other, the chromatin material undergoes the formation of chromosomes, the nuclear membranes disappear, and the first division of the zygote occurs.

The penetration of the male nucleus into the female nucleus of *Fossombronia* has not been observed. The fusion nucleus shows the two masses quite distinct in the nuclear cavity.

In *Pellia* the male nucleus comes in contact with the female nucleus, whereupon the membrane of the latter disappears at the point of contact. A new membrane which is formed about the male nucleus is continuous with the female nucleus, and as a result the two nuclei are surrounded by a common membrane.

Fusion occurs, and the fusion nucleus is distinguished by the presence of two nucleoli.

In *Riccardia* the male nucleus penetrates endwise by piercing the membrane. The passage of material into the female nucleus is very slow. The two masses of chromatin are quite distinct, each occupying separate regions of the nuclear cavity, but these become optically indistinguishable before division is initiated.

The situation as described in *Funaria* offers a good many points of contrast. Penetration of the male nucleus into the female nucleus occurs very shortly after insemination. The process of penetration is similar to that in *Riccardia*, but the behavior of the two masses of chromatin is distinctly different in the two cases. In *Funaria* there is a very definite fusion of the two masses with the disappearance of the nuclear membrane. The fusion nuclear body, resulting from the coalescence of the male nucleus and the condensed reticulum of the egg, remains very distinct and definite in the central region of the egg, but is for a time not delimited by any perceptible membrane. The reappearance of the membrane some time after fusion is another point of contrast, and the fact that the chromatin material, at the inception of the prophase of the first mitosis, is distinguished at the periphery of the fusion nuclear cavity places *Funaria* in a category by itself, as far as fertilization is concerned. This particular method, distinct and unique, has not been described for any species of plant.

The fact that in all Bryophytes previously studied there is a prolonged period of time before the fusion of the two nuclei is interesting. There seems to be a very definite period in the Liverworts during which the male and female nuclei retain their identity. In *Funaria* penetration of the male nucleus into the female nucleus is followed shortly by their fusion. However, the fusion body remains in the cytoplasm of the egg for some time before mitosis occurs. Hence, the two-celled embryo is encountered in *Funaria* about the same number of hours after flooding as it is in *Sphaerocarpos*.

Rickett ('23) finds that the length of time required in *Sphaerocarpos* for visible sporophytic development is from two to eight weeks. However, in *Funaria* the length of time required for mature sporophytic development is five weeks.

The ferns, which also belong to the group of plants known as Archegoniates, have distinct methods of nuclear fusion. The processes of nuclear fusion which have been described in the ferns do not present any points of similarity to the process found in *Funaria*.

Table II represents in a condensed form the more essential points of contrast between the fertilization processes of *Funaria* and the other members of the Bryophytes which have been studied.

The presence of a mucilaginous plug in the neck of the archegonium of *Funaria* is another characteristic restricted apparently to this particular group of plants, although no other moss has been studied adequately. No record has been found of any previous mention of this plug. Rickett ('23) considers the mucilaginous material present in the cavity of the venter and the neck in *Sphaerocarpos* as analogous to the "fertilization membrane" described by other workers. The mucilaginous plug observed in *Funaria* is not comparable in structure and location with the mucilaginous material of *Sphaerocarpos*, although the latter may be similar in origin and function. In the first place, there is no indication of any such substance being present in the canal of any archegonium in which insemination has not occurred. In the second place, this mucilaginous plug is always associated with the two tiers of cells of the neck adjoining the venter, never with any other cells. In 334 archegonia examined with reference to this particular point, 219, or 65½ per cent, showed this mucilaginous plug to be present. Some of the archegonia studied were sectioned transversely; hence, if the plug were present it would not be visible in the same sections as the eggs (but the sections through that part of the neck might be expected to show it). It is entirely possible that the percentage would be much higher if all the material had been sectioned longitudinally. In all of these archegonia in which the mucilaginous plug is present it is associated with the two tiers of cells of the neck adjoining the venter. Never do any other than these two tiers of cells secrete the mucilaginous material, and hence it is inferred that this plug is a secretion of these cells but its function is as yet undetermined. If the plug were analogous to a fertili-

TABLE II

COMPARISON OF BRYOPHYTES WITH REGARD TO FERTILIZATION

	Sphaerocarpos	*Fossombronia*	*Pellia*	*Riccardia*	*Funaria*
Size of egg	40 x 20 μ	25 μ	?	25 μ	7.7 μ
Size of nucleus	13 x 10 μ	10 μ	10 μ	10 μ	2.9 μ
Penetration of male nucleus into egg	Not known	Pierces membrane	Pierces membrane	Pierces membrane	Pierces membrane
Time required for penetration	15 minutes	0–6 minutes	20–40 minutes	20–40 minutes	1 hour, 35 minutes
Penetration of male nucleus into female nucleus	No penetration, two nuclei come in contact	Not known	No penetration, fusion in reticulate condition	Endwise	Endwise
Role of nuclear membrane	Disappears	Remains	Disappears at point of contact then reappears	Remains	Disappears
Behavior of chromatin	Organized into chromosomes	Not known	Not definite	Becomes dispersed	Two bodies fuse as such
Time elapsed before fusion	44 hours	40–60 hours	24–36 hours	26–36 hours	10 hours, 30 minutes
First division of nucleus (two-celled embryo)	73 hours, 45 minutes	168 hours	144–168 hours	144–168 hours	70–93 hours

zation membrane its function would be to prevent the penetration of any supernumerary antherozoids into the egg. The presence of a relatively large number of antherozoids within the ventral cavity, after penetration of one antherozoid, makes this assumption doubtful. The fact that an unusually large number of antherozoids appears to have accumulated above the plug gives one the impression that the plug prevents further entrance of antherozoids into the cavity. It may also be conceived to afford protection against injury to the zygote, preventing evaporation of water or entrance of bacteria.

There is no indication of any fusion between the ventral canal cell and the egg cell, such as has been described by Bryan ('20) in the case of *Sphagnum*. The egg cell in *Funaria* is approximately three times the size of the ventral canal cell. The ventral canal cell, as it disintegrates, decreases in size until it is scarcely perceptible above the egg cell and then disappears completely. The rounding up of the egg cell and decrease in size of the two cells leaves a space between the two cells. This space makes it evident that the cells do not come in contact with each other, thus eliminating the possibility of fusion.

The structures observed in the cytoplasm of the egg, in the fixations which were made approximately forty-five hours after flooding, have been definitely determined to be plastids. Throughout the development of the fertilized egg, at various periods after flooding, rod-like structures and granules have been observed in the cytoplasm of the egg, but this particular lot is the only material in which definite plastids have been identified. The plastids present the same vacuolated appearance and are similar in structure to those found in other tissues of the plant except that they are smaller. Sapehin ('13) finds plastids in the egg of *Bryum*. From his drawing, however, it is very evident that the egg has just been separated from the ventral canal cell, since it does not display the definitely rounded appearance associated with mature eggs. The plastid-like bodies appear to be small, spherical, and relatively few in number. In addition to these small spherical structures Sapehin represents definite rods and minute granules. These latter structures in *Bryum* are very similar to those which are found in eggs of *Funaria* three hours and thirty minutes after insemination.

Showalter ('27b) includes a drawing of *Pellia* showing very definite starch granules. These appear in a mature egg which contains two male nuclei, but in which no nuclear fusion has occurred. Showalter ('28) has observed in eggs of *Riccardia pinguis*, type C, inseminated with antherozoids of *Riccardia pinguis*, type B, that plastids with starch grains were sometimes quite conspicuous. Motte ('28) figures very definite rods, together with some irregularly shaped bodies in the cytoplasm of the egg of *Hylocomium*. He has used the particular technique which has been developed for the study of plastids. He assumes that these irregularly shaped bodies are plastids, and considers them, inasmuch as the archegonium is advanced in age, to be plastids which develop as a result of non-fertilization of the egg and to be forerunners of cellular death. In *Funaria hygrometrica* he finds no indication of plastids in the cytoplasm of an egg that is somewhat past the mature stage.

The fixations of *Funaria flavicans* were made for other purposes than the studying of plastids, but it is interesting to note that these rod-like structures and spherical bodies are present in the cytoplasm of the egg regardless of the killing fluid or the stain used. The fluid which brought out the definite plastid bodies was that of Benda, and in other lots of material fixed in Benda's fluid no plastids, rods, or granules were observed in the cytoplasm. The rod-like structures and granules, which have been observed, are present in those eggs which show very clearly the male nucleus in contact with the female nucleus. In the later stage in which the definite plastids are observed, the presence of the mucilaginous plug would indicate that insemination had taken place. It is questionable whether nuclear fusion has occurred, since the presence of these bodies makes it somewhat difficult to determine the nuclear structure. Not all of the eggs of this particular lot show these plastids, and it is possible that they have developed because of non-fertilization. The very scanty amount of evidence prevents one from making any definite inferences regarding these plastids.

The condition of the nucleus with reference to the condensed chromatin shows some definite points of similarity to those described by Showalter ('28) for *Riccardia*. He observes that,

after penetration of the male nucleus into the cytoplasm of the egg, the chromatin of the female nucleus condenses into a compact mass about the nucleolus, leaving a region in which there is no staining substance present. In early stages the chromatin is readily distinguished about the nucleolar body. In *Funaria*, however, the region about the nucleolus is very dense in appearance and does not seem to display any of the details characteristic of true chromatin. This dense mass gradually disappears with the entrance of the male nucleus into the female nucleus, leaving a region in which there is no staining substance present. No measurements were made of the nucleolar body to determine if there was an increase in its size, which at all times is extremely minute, making detailed observations difficult. It seems logical, however, to assume that the nucleolus of the egg nucleus in *Funaria* is in reality a condensed mass of chromatin enclosing the true nucleolus and is imbedded in some cytoplasmic material which undergoes structural changes with the occurrence of fertilization.

Cytokinesis has not been thoroughly investigated in the Mosses. Wilson ('09) depicted very definitely division by cell-plate formation in *Mnium*. Allen ('16) found that the spore-mother-cell of *Catharinea* presented a lobed appearance and stated that this was the first observed occurrence of lobing in the Bryales. The lobing may be interpreted as furrows which grow in dividing the spore-mother-cell into tetrads. The method of division in *Funaria* is doubtless that of cell-plate formation. The definite thickenings which appear at the equator between the poles and which grow out toward the periphery of the cell are regarded as incipient cell-plates.

Summary

1. Sporelings, obtained from spores of *Funaria flavicans* Michx. sown on sterile soil, were grown under controlled conditions. At the time when the archegonia and antheridia were mature, insemination was brought about by flooding the cultures. Fixations were made at intervals after flooding and the material studied microscopically.

2. The volume of the egg of *Funaria* was found to be approximately one-eighteenth that of the egg of *Riccardia*. The

volume of the nucleus of *Funaria* was found to be one-fortieth that of *Riccardia*.

3. The antherozoid penetrates the cytoplasm of the egg by a gradual process, and takes place, for the most part, at the basal end.

4. The male nucleus, having assumed a spherical form, comes in contact with the female nucleus and then passes into the nuclear cavity.

5. The region about the condensed chromatin of the female nucleus is very clear, whereas the region about the male nucleus is chromophyllic.

6. After penetration of the male nucleus the nuclear membrane about the female nucleus becomes irregular in outline and disappears, leaving the condensed chromatin and the male nucleus in the center of the nuclear cavity.

7. The condensed chromatin of the female nucleus gradually fuses with the male nucleus. After this fusion has occurred, a nuclear membrane reappears around the fusion nucleus.

8. In connection with fertilization, a mucilaginous plug is developed in the neck of the archegonium. It is thought to be a secretion of the first two tiers of neck cells above the venter, since it is always found associated with these cells.

9. No fusion of the egg cell with the ventral canal cell, such as that reported in *Sphagnum* by Bryan, was observed.

10. Cytokinesis of the spore-mother-cell is by cell-plate formation.

11. These results, the only ones so far obtained in connection with fertilization in Mosses, are compared with those in other Bryophytes.

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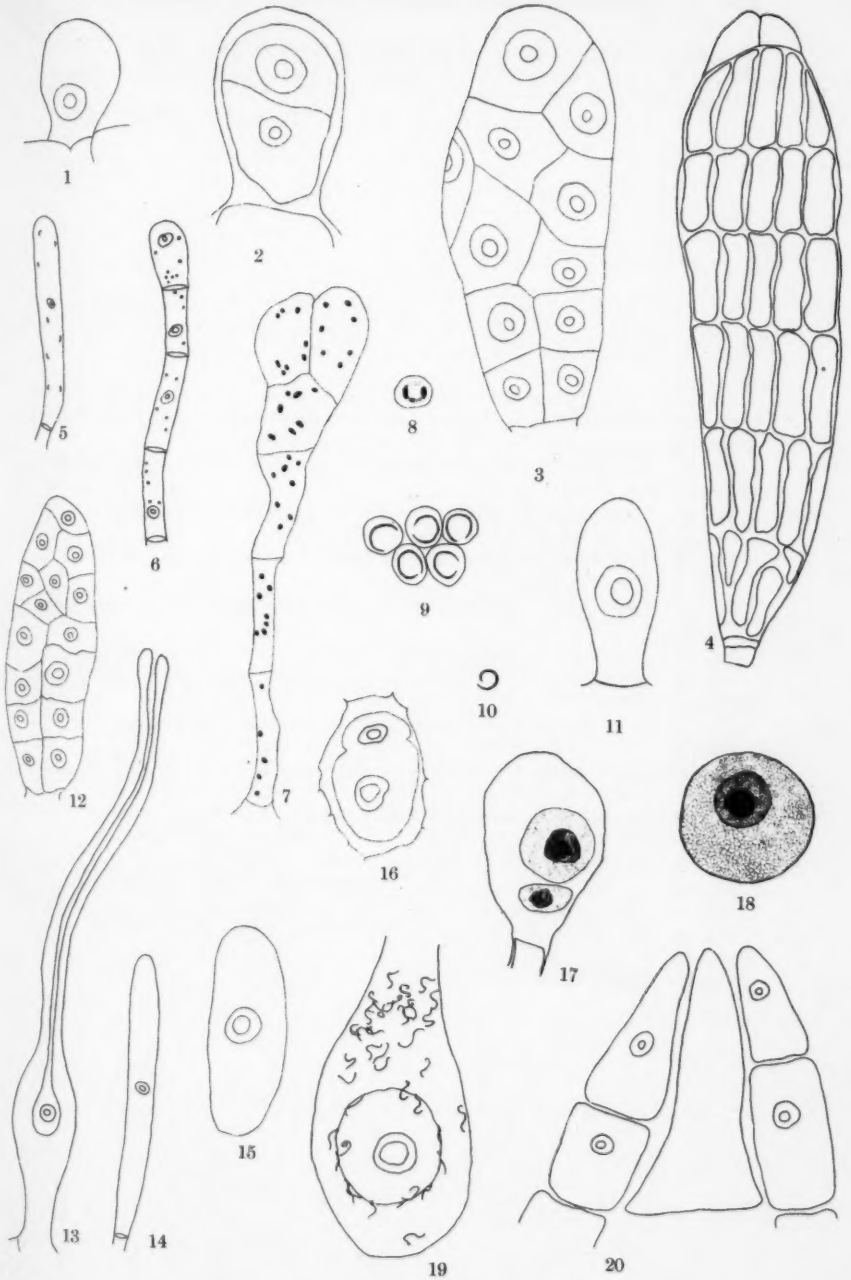
Explanation of Plate

PLATE 43

All figures were drawn with the aid of the camera lucida at the magnification indicated.

Figs. 4, 5, 6, 9, 10 were drawn from living material. Figs. 1, 2, 3, 7, 8, 11–57 were drawn from stained preparations.

Fig. 1. Single-celled antheridium. × 1500.
Fig. 2. Two-celled antheridium. × 1500.
Fig. 3. Multicellular antheridium. × 1500.
Fig. 4. Mature antheridium. × 380.
Fig. 5. Single-celled paraphysis. × 380.
Fig. 6. Several-celled paraphysis. × 380.
Fig. 7. Mature paraphysis. × 380.
Fig. 8. Intranuclear division. × 1500.
Fig. 9. Mature antherozoids in gelatinous envelope. × 750.
Fig. 10. Sperm—cilia not visible. × 750.
Fig. 11. Single-celled archegonium. × 1500.
Fig. 12. Several-celled archegonium. × 1500.
Fig. 13. Mature archegonium. × 150.
Fig. 14. Paraphysis of archegonial head. × 380.
Fig. 15. Cell before egg and ventral canal cell have been cut off. × 1500.
Fig. 16. Cell showing invagination of cytoplasm. × 750.
Fig. 17. Venter with degenerating ventral canal cell. × 750.
Fig. 18. Mature egg immediately after flooding. × 1500.
Fig. 19. Egg being surrounded by antherozoids, 35 min. after flooding. Diagrammatic × 1500.
Fig. 20. Mucilaginous plug. × 1500.

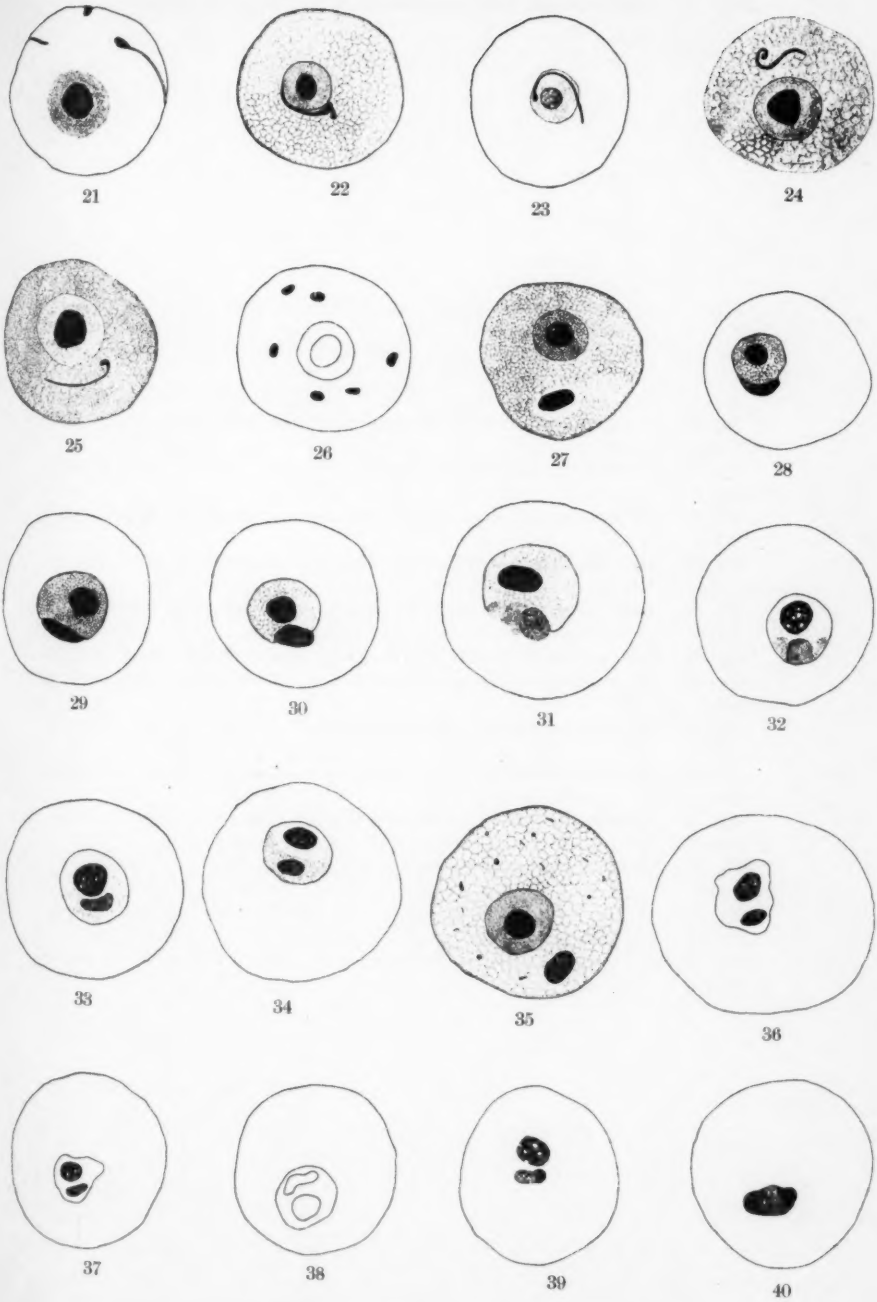


BEARDSLEY—FUNARIA FLAVICANS

Explanation of Plate

PLATE 44

Fig. 21. Antherozoids adpressed to egg, 1 hr. 35 min. after flooding. × 1500.
Fig. 22. Antherozoids adpressed to egg, 2 hr. 20 min. after flooding. × 1500.
Fig. 23. Antherozoid in process of penetration, 1 hr. 35 min. after flooding. × 1500.
Fig. 24. Antherozoid in cytoplasm of egg, 2 hr. 20 min. after flooding. × 1500.
Fig. 25. Antherozoid in cytoplasm of egg, 2 hr. 20 min. after flooding. × 1500.
Fig. 26. Egg showing polyspermy, 1 hr. 35 min. after flooding. × 1500.
Fig. 27. Antherozoid assuming a spherical form, 3 hr. 30 min. after flooding. × 1500.
Fig. 28. Antherozoid coming in contact with egg nucleus, 4½ hr. after flooding. × 1500.
Fig. 29. Male nucleus in contact with female nucleus, 3½ hr. after flooding. × 1500.
Fig. 30. Male nucleus in contact with female nucleus, 3½ hr. after flooding. × 1500.
Fig. 31. Male nucleus penetrating female nucleus, 4½ hr. after flooding. × 1500.
Fig. 32. Egg nucleus showing vacuolation of condensed chromatin, 4½ hr. after flooding. × 1500.
Fig. 33. Male nucleus within female nuclear membrane, 4½ hr. after flooding. × 1500.
Fig. 34. Male nucleus within female nuclear membrane, 4½ hr. after flooding. × 1500.
Fig. 35. Egg showing rod-like bodies in cytoplasm, 2 hr. 20 min. after flooding. × 1500.
Fig. 36. Membrane becoming irregular, 5½ hr. after flooding. × 1500.
Fig. 37. Membrane becoming irregular, 5½ hr. after flooding. × 1500.
Fig. 38. Membrane becoming irregular, 6½ hr. after flooding. × 1500.
Fig. 39. Disappearance of membrane, 6½ hr. after flooding. × 1500.
Fig. 40. Maternal and paternal chromatin in process of fusion, 6½ hr. after flooding. × 1500.

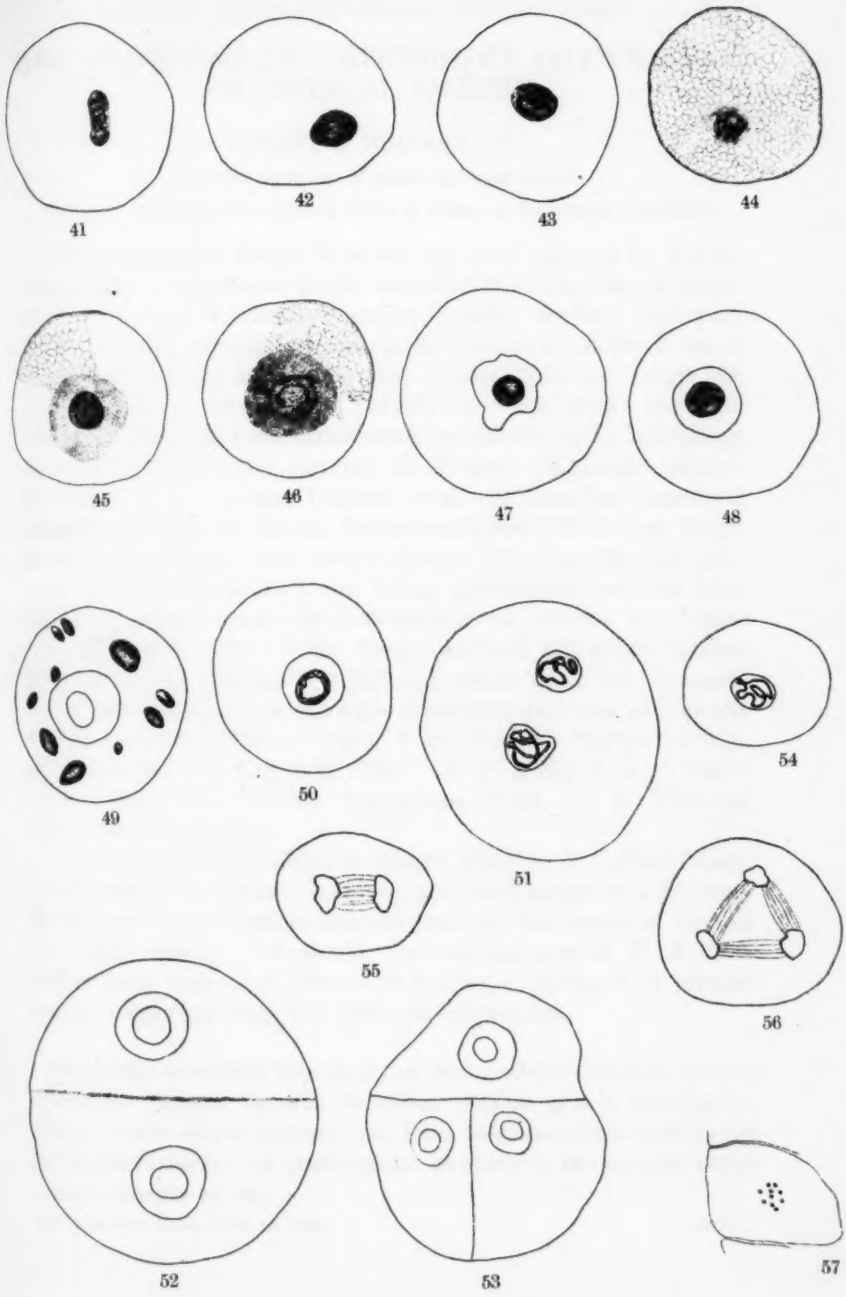


BEARDSLEY—FUNARIA FLAVICANS

Explanation of Plate

PLATE 45

Fig. 41. Maternal and paternal chromatin in process of fusion, 7½ hr. after flooding. × 1500.
Fig. 42. Fusion body, 14 hr. after flooding. × 1500.
Fig. 43. Fusion body, 14 hr. after flooding. × 1500.
Fig. 44. Cytoplasm becoming dense around fusion body, 14 hr. after flooding. × 1500.
Fig. 45. Cytoplasm denser, 16 hr. after flooding. × 1500.
Fig. 46. Cytoplasm denser, 18 hr. after flooding. × 1500.
Fig. 47. Irregular membrane, 22 hr. 20 min. after flooding. × 1500.
Fig. 48. Definite membrane, 48 hr. 45 min. after flooding. × 1500.
Fig. 49. Egg showing presence of plastids, 45 hr. 20 min. after flooding. × 1500.
Fig. 50. Egg showing dispersal of chromatin about periphery of fusion body, 93 hr. after flooding. × 1500.
Fig. 51. Binucleate embryo, 93 hr. after flooding. × 1500.
Fig. 52. Embryo showing nuclei in resting stage, 93 hr. after flooding. × 1500.
Fig. 53. Three-celled embryo, 93 hr. after flooding. × 750.
Fig. 54. Spore-mother-cell. × 1500.
Fig. 55. Spore-mother-cell during first division. × 1500.
Fig. 56. Spore-mother-cell during second division. × 1500.
Fig. 57. Chromosomes in archegonium. × 1500.



BEARDSLEY—FUNARIA FLAVICANS

NEW OR OTHERWISE NOTEWORTHY APOCYNACEAE OF TROPICAL AMERICA

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Tabernaemontana Killipii Woodson, sp. nov., arborea ca. 4–5 m. alta; ramulis dichotome divisis teretibus in sicco obscure angulatis junioribus brevissime scabro-hirtellis tandem glabratis; foliis oppositis petiolatis subcoriaceis late ovato-ellipticis apice breviter obtuseque acuminatis basi obtusis 25–30 cm. longis 14–15 cm. latis omnino glabris; petiolis ca. 1 cm. longis basi imo fossatis marginibus linea transversali conjunctis; cymis lateralibus divergenter dichotome ramosis 20–30-floris pedunculo scabro-hirtello 5–6 cm. longo; bracteis ovato-reniformibus scariaceis margine ciliolatis ca. 0.1 cm. longis; pedicellis 0.75–1.0 cm. longis glaberrimis; calycis lobis ovato-oblongis obtusiusculis plus minusve inaequalibus 0.3–0.4 cm. longis glaberrimis; corollae lobis oblique oblongis paulo dolabriformibus ca. 0.3–0.4 cm. longis tubo cylindrico 0.75–1.0 cm. longo; antheris linearibus omnino insertis; ovariis oblongoideis glabris nectario nullo vel vix manifesto; folliculis laevibus oblongo-ellipsoideis utrinque acuminatis divergentibus parvulis.—"Peru, Dept. Loreto: Iquitos, woods, alt. about 100 m., Aug. 2–8, 1929." *E. P. Killip & A. C. Smith 27414* (Mo. Bot. Garden Herbarium, TYPE, U. S. National Herbarium, duplicate).

Tabernaemontana Killipii is closely allied to *T. hirtula* Mart. The leaves of the former, however, are much larger, of a different shape, and with a petiole scarcely one-half the length of that of the latter species. Moreover, the inflorescence of *T. Killipii* differs from that of *T. hirtula* in having a peduncle of greater length, longer pedicels, and glabrous calyx-lobes.

Rauwolfia lauretiana Woodson, sp. nov., arborea omnino glabra altitudine ignota; ramulis teretibus cortice griseis longitudinaliter striatis sparse lenticellosis; foliis membranaceis longiuscule petiolatis ternatim vel quaternatim in apicibus ramulorum verti-

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cillatis ovatis apice longe obtuseque acuminatis basi subiter attenuatis conspicue inaequalibus maioribus 9–10 cm. longis ca. 5 cm. latis minoribus 5–7 cm. longis 3.0–3.5 cm. latis petiolo 1.5–2.0 cm. longo eglanduloso; cymis terminalibus 4–12-floris divergentibus pedunculo ca. 1 cm. longo; bracteis squamosis minimis; pedicellis pedunculis subaequantibus; calycis lobis late deltoideis obtusissimis ca. .075 cm. longis 0.2–0.25 cm. latis margine minute ciliolatis intus eglandulosis; corollae tubo cylindrico basi haud dilatato ca. 1 cm. longo fauce ca. 0.2 cm. diametro extus glabro intus sub staminibus sparse piloso lobis obovatis obtusiusculis ca. 0.5 cm. longis; antheris ovoideis ca. 0.2 cm. longis apice haud appendiculatis; ovariis oblongoideis glabris nectarium annuliforme apice integrum ca. triplo superantibus; fructibus ignotis.—"Peru, Dept. Loreto: Mishuyacu, near Iquitos, alt. 100 meters; forest, Nov., 1929." *G. Klug 35* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Taking Markgraf's recent revision of the genus *Rauwolfia*[1] as a guide, it has been ascertained that *R. lauretiana* should be classified as included within the section *Grandiflorae* Mgf., and is evidently most closely related to *R. paucifolia* A. DC. From that species, however, *R. lauretiana* presents a strong contrast by reason of the leaves, which are lanceolate, 2.5–4.5 cm. long, with a petiole about 0.5 cm. long in the former species, and are ovate, 5–10 cm. long, and borne upon a petiole 1.5–2.0 cm. long in the latter. The dimensions of the floral organs are also different, the calyx-lobes of *R. paucifolia* being lanceolate, and those of *R. lauretiana* extremely depressed-deltoid. The corolla tube of the latter species, moreover, is nearly one-third longer than that of the former.

Rauwolfia sanctorum Woodson, sp. nov., arborea omnino glabra ca. 3–4 m. alta; ramis pendulis teretibus rimosis olivaceo-griseis; foliis petiolatis subcoriaceis ternato-verticillatis paene inaequalibus elliptico-oblanceolatis apice longe et acute acuminatis basi in petiolum eglandulosum 1.0–1.5 cm. longum cuneato-angustatis cum petiolo 9–14 cm. longis 3.5–4.0 cm. latis supra

[1] Mgf. in Fedde, Repert. 20: 114–122. 1924.

nitidulis subtus pallidioribus nervis secundariis utrimque prominulis arcuatis sat remotis; cymis solitariis evidenter terminalibus dichasialibus divergentibus 8–12-floris pedunculo gracili ca. 5 cm. longo; pedicellis ca. 0.5 cm. longis; bracteis squamosis subulatis minimis; calycis lobis ovatis breviter acuminatis ca. 0.1 cm. longis vix aequalibus; corollae gilvae tubo longe cylindrico 1.25–1.5 cm. longo 0.15 cm. diametro metiente sub fauce paulum inflato extus glabro intus in dilatatione superiore barbato-piloso lobis obovato-oblongis obtusissimis ca. 0.4 cm. longis; antheris ovoideis longe acuminatis subsessilibus; stigmate late tympaniformi ca. 0.1 cm. alto apice obtuse bilobato basi annulo conspicuo ornato; ovariis obovoideis glabris nectarium breviter cylindricum duplo superantibus; fructibus ignotis.—"Colombia, Dept. Santander: northern slope of Mesa de los Santos; alt. 1000–1500 m., Dec. 11–15, 1926." *E. P. Killip & A. C. Smith 15392* (Mo. Bot. Garden Herbarium, TYPE, U. S. National Herbarium, duplicate).

Like the species immediately preceding, *Rauwolfia sanctorum* appears to be most definitely related to the species of the section *Grandiflorae*. It is a small tree bearing ternately verticillate, elliptic-oblanceolate, definitely petiolate leaves of a somewhat leathery texture. The calyx-lobes are one-twelfth to one-fifteenth the length of the corolla-tube, which is 1.25–1.5 cm. long. The terminal cyme is solitary. On the other hand, the leaves of *R. bahiensis* A. DC., which is evidently its nearest affinity, are obovate, the calyx-lobes are about one-third the length of the corolla-tube, which is only about 0.8 cm. long, and the terminal cymes are geminate or ternate. The geographical distribution of either species is also distinct.

Dipladenia Achrestogyne Woodson, sp. nov., suffruticosa volubilis pauce ramosa omnino glabra; ramis gracilibus teretibus in sicco plus minusve striatis; foliis oppositis petiolatis membranaceis late ovato-oblongis apice breviter acuminatis basi obtusiusculis 5–9 cm. longis 2.5–6.0 cm. latis in sicco fuscis subtus in parenchymate inter venulas levissimis pallidis; racemis alternatis lateralibus subterminalibusve subspiciformibus ca. 5–15-floris pedunculo foliis fere semper aequante; bracteis scariaceis ovato-lanceolatis 0.5–0.7 cm. longis pedicellos paulo superantibus; lobis calycis

scariaceis anguste lanceolatis acutiusculis 0.3–0.4 cm. longis basi intus multiglandulosis; corollae lobis oblique oblongis plus minusve dolabriformibus 0.75–1.0 cm. longis paulo reflexis tubo longe-cylindrico 1.5–1.75 cm. longo; ovariis oblongoideis glabris nectario 2–5-lobo vix manifesto; folliculis ignotis.—"Colombia, Dept. Cundinamarca: rocky canon, Chapinero, near Bogota, alt. 2800–2900 m., Sept. 18–23, 1917." *F. W. Pennell 2034* (N. Y. Bot. Garden Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Dipladenia Achrestogyne is easily distinguished from *D. congesta*, which should evidently be regarded as its closest affinity, since it is absolutely glabrous in all parts, whereas the latter species is softly tomentulose to hirtellous throughout. The inflorescence of the former, moreover, is a regular, subspiciform raceme with pedicels rather laxly and distantly arranged, differing markedly from the distally congested inflorescence of *D. congesta*.

D. Achrestogyne has been named from the Greek ἄχρηστος and γυνή, with reference to the extreme reduction of the gynoecial nectaries and to the theory that they may be regarded as carpellodes.

Dipladenia oblongifolia Woodson, sp. nov., suffruticosa volubilis plus minusve ramosa; ramis gracilibus flexuosis glabris; foliis oppositis petiolatis membranaceis late oblongis apice breviter acuminatis basi obtusiusculis 7–15 cm. longis 2.0–3.5 cm. latis supra glabris subtus tenuissime puberulis petiolo 2–3 cm. longo in annulo obscuro stipularum instructo; racemis lateralibus alternatis 3–5-floris pedunculo foliis subaequante; bracteis scariaceis minimis; pedicellis ca. 1 cm. longis; calycis lobis scariaceis anguste lanceolatis 0.5–0.7 cm. longis basi intus multiglandulosis; corollae lobis obovatis dolabriformibusque ca. 3.5 cm. longis paulo reflexis tubo 4.0–4.5 cm. longo usque 1/3 longitudinem anguste cylindrico dein latius cylindrico-dilatato fauce ca. 1.25 cm. lato; nectario 2-lobo ovariis bis vel ter breviore; folliculis ignotis.—"Bolivia, Sur-Yungas: La Florida, vec. de Yanocochi, alt. 1700 m., Dec. 6, 1906." *O. Buchtien 590* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Dipladenia oblongifolia finds its natural alliance with the group of species centering about *D. Martiana*, because of its volubile habit and conspicuous nodal, or stipular appendages. It may easily be distinguished from *D. glabra* Rusby, of the same general region of South America, by its characteristically oblong foliage which is softly puberulent beneath, and by its larger flowers which are also of a somewhat different construction in general. The squamellae, or internal glandular emergences of the calyx, moreover, are decidedly fewer in the latter species, and are arranged in groups alternate with the lobes of the calyx, whereas they are more numerous and are uniformly distributed in *D. oblongifolia*.

Dipladenia upatae Woodson, sp. nov., suffruticosa volubilis pauce ramosa omnino glabra; ramis gracilibus flexuosis in sicco plus minusve striatis; foliis oppositis petiolatis membranaceis oblongo-lanceolatis apice acuminatis basi late cordatis evidenter conduplicativis 7–12 cm. longis 2.0–3.5 cm. latis petiolo 0.5–0.75 cm. longo in annulo obscuro stipularum instructo; racemis lateralibus alternatis ca. 3-floris pedunculo foliis subaequante; bracteis scariaceis minimis; pedicellis ca. 0.5 cm. longis; calycis lobis scariaceis glabris lanceolatis acuminatis 0.4–0.5 cm. longis basi intus biglandulosis; corollae lobis late obovato-dolabriformibus ca. 2.5 cm. longis paulo reflexis tubo 2.5–3.0 cm. longo usque 1/2 longitudinem anguste cylindrico dein paulo latiore fauce ca. 0.5 cm. diametro; nectario 2-lobo ovariis oblongoideis glabris bis vel ter breviore; folliculis ignotis.—"Venezuela: Upata," date lacking, *E. Osta 1014* (Herbarium Mus. Hist. Nat. Vindobonensis, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

D. upatae falls naturally into the group of species of *Dipladenia* comprising *D. fragrans* A. DC., *D. urophylla* Hook., and *D. surinamensis* Pulle, all of which are characterized by leaves which are more or less conduplicate when desiccated. From all the close relatives to which reference has been made, however, *D. upatae* differs by reason of the extremely narrow, cylindrical corolla-throat, and in the paired squamellae, or internal calycine emergences, which are indefinite in number among the allied species.

Odontadenia cognata (Stadelm.) Woodson, n. comb.
Echites cognata Stadelm. Flora **24**: I Beibl. 79. 1841.
Anisolobus cognatus (Stadelm.) Muell.-Arg. in Martius, Fl. Bras. **6**[1]: 113. 1860.

Odontadenia Perrottetii (A. DC.) Woodson, n. comb.
Anisolobus Perrottetii (A. DC.) in DC. Prodr. **8**: 395. 1844.

Odontadenia polyneura (Urb.) Woodson, n. comb.
Rhabdadenia polyneura Urb. Symb. Ant. **7**: 337. 1912.

Odontadenia Killipii Woodson, sp. nov., fruticosa volubilis omnino glabra; ramis ramulisque teretibus fuscis lenticellas parvas conspicue gerentibus; foliis oppositis longiuscule petiolatis subcoriaceis in sicco fuscis late oblongo-ellipticis apice subiter et obtuse acuminatis basi acutiusculis 6–8 cm. longis 3–5 cm. latis petiolo 1.0–1.5 cm. longo in annulo obscuro stipularum instructo; cymis terminalibus 15–20-floris pedunculo petiolos 5–6-plo superante; bracteis ovatis squamosis 0.2–0.3 cm. longis; pedicellis 0.5–0.7 cm. longis; calycis lobis plus minusve inaequalibus ovatis vel late ovato-oblongis 0.2–0.3 cm. longis intus in marginibus positis 1–2 glandulis; corollae speciosae lobis oblique obovatis dolabriformibus 2.5–3.0 cm. longis paulo reflexis tubo 3.0–3.5 cm. longo usque 1/4 altitudinem anguste cylindrico dein staminigero et abrupte conico-dilatato; antheris linearibus dorso minute puberulo-papillatis 0.3–0.4 cm. longis; nectario tubuloso-cupuliformi apice integro vel minutissime crenulato ovariis oblongoideis glabris paulo breviore; folliculis ignotis.—"Peru, Dept. Loreto: Iquitos; woods, alt. about 100 m., Sept. 26, 1929." *E. P. Killip & A. C. Smith 29847* (Mo. Bot. Garden Herbarium, TYPE, U. S. National Herbarium, duplicate).

Odontadenia Killipii bears a strong superficial resemblance to *O. cognata*, chiefly because both species have a terminal inflorescence and foliage which assumes a decided bronze coloration upon desiccation. The coloration of the desiccated foliage, incidentally, appears to be a trustworthy and quickly perceived indicator of relationship among the species of the genus, although it has evidently never been used as such in publication. *O.*

Killipii may easily be distinguished from *O. cognata*, since the former has a corolla-tube 3.0–3.5 cm. long, narrowly cylindrical for one-quarter its length and then abruptly and broadly conical, calyx-lobes which are among the shortest of the genus, only 0.2–0.3 cm. long, and oblong-elliptic leaves which are acute or somewhat obtuse at the base; whereas the latter has a corolla-tube 5–6 cm. long, narrowly cylindrical for about one-half its length and then abruptly dilated into a much broader, cylindrical throat, calyx-lobes 0.8–0.9 cm. long, and broadly ovate-cordate leaves with a broadly auriculate base.

Odontadenia Sandwithiana Woodson, sp. nov., fruticosa volubilis omnino glabra; ramis ramulisque teretibus plus minusve longitudinaliter striatis lenticellas parvas conspicue gerentibus; foliis oppositis longiuscule petiolatis subcoriaceis in sicco olivaceis oblongo-ellipticis apice breviter et saepius obtuse acuminatis basi acutis et paulo inaequilateralibus 10–15 cm. longis 4–6 cm. latis superne angustiore petiolo 1.5–2.0 cm. longo in annulo obscuro stipularum instructo; cymis lateralibus vel pseudoterminalibus 6–10-floris pedunculo petiolos ca. duplo superante; bracteis ovatis squamosis 0.1–0.2 cm. longis; pedicellis 0.2–0.3 cm. longis; calycis lobis oblongis obtusis distinctissime inaequalibus 1.0–1.5 cm. longis intus in marginibus positis 1–2 glandulis; corollae lobis late et oblique oblongis in alabastro ca. 1 cm. longis tubo ca. 2 cm. longo usque 1/2 altitudinem anguste cylindrico dein staminigero et paulo ampliore cylindrico-dilatato; antheris linearibus dorso glabris vel minutissime papillatis 0.5–0.6 cm. longis; nectario tubuloso apice crenulato ovarium oblongoideum glabrum paulo superante; folliculis ignotis.—"British Guiana, Essequibo River: Moraballi Creek, near Bartica, alt. near sea-level, Nov. 2, 1929." *N. Y. Sandwith 552* (Herbarium Kew., TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Odontadenia Sandwithiana is closely allied to *O. Perrottetii*, indigenous to the same general region, but differs from the latter in its axillary or pseudoterminal inflorescence, its smaller corolla with a cylindrical, not campanulate, proper throat, and in its much larger leaves borne upon a petiole more than twice as long as that of the latter species. Although fully mature flowers were

not available for study, buds which were collected shortly before unfolding are of dimensions unusually small for the genus.

Odontadenia stemmadeniaefolia Woodson, sp. nov., fruticosa volubilis; ramis teretibus longitudinaliter striatis glabris in sicco dilute flavo-coloratis; foliis oppositis breviuscule petiolatis rigidule membranaceis vel subcoriaceis fuscentibus oblongo-obovatis basi saepissime plus minusve cuneato-angustatis apice breviter et obtuse cuspidatis 15–20 cm. longis 8–10 cm. latis superne angustioribus omnino glabriusculis petiolo 1.0–1.5 cm. longo in annulo obscuro stipularum instructo; cymis lateralibus 3–5-floris pedunculo foliis paulo breviore; bracteis squamosis minimis; pedicellis 1.0–1.25 cm. longis glabris; calycis lobis subaequalibus ovato-reniformibus ca. 0.2 cm. longis glabris vel margine minutissime ciliolatis intus in margine positis 2–3 glandulis; corollae lobis oblique et late obovatis 1.5–2.0 cm. longis tubo ca. 1.5 cm. longo extra calycem ventricoso-dilatato dein constricto et ad basin partis iterum dilatatae staminigero et sensim obconico-dilatato; antheris anguste oblanceolatis dorso dense lanulosis ca. 0.8 cm. longis; ovariis ovoideis glabris nectario cupulato apice crenulato et multifido subaequante; folliculis ignotis.—"Peru, Dept. Loreto: Mishuyacu, near Iquitos, alt. 100 m.; forest, Jan., 1930." *G. Klug 782* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

The nearest relative of the preceding species is evidently *O. speciosa* Benth., as is witnessed by the large, obovate and somewhat cuneate foliage and the short, obconic corolla-throat. The corolla of *O. stemmadeniaefolia*, however, is only about one-half to one-third the size of that of *O. speciosa*, and also differs in the color, which is said by the collector to have been "brick red." Additional features which may be cited as distinguishing *O. stemmadeniaefolia* from *O. speciosa* are the smaller, more nearly isophyllous calyx and the fewer-flowered inflorescence with the pedicels congested at the end of the longer peduncle. The specific name refers to the resemblance of the foliage to that of several species of *Stemmadenia*.

Odontadenia augusta Woodson, sp. nov., fruticosa volubilis; ramis teretibus lenticellas parvas conspicue gerentibus glabris

in sicco rubidulo-coloratis; foliis oppositis longiuscule petiolatis rigidule membranaceis late oblongis apice breviter et obtuse acuminatis basi obtusis superne acutiusculis 20–25 cm. longis, 8–10 cm. latis omnino glabriusculis supra fuscentibus subtus olivaceo-viridibus petiolo ca. 2 cm. longo minute hispidulo in annulo obscuro stipularum instructo; paniculis lateralibus 15–20-floris pedunculo foliis paulo breviore minute ferrugineo puberulo; bracteis ovatis squamosis minimis; pedicellis 1.0–1.25 cm. longis sicut ad pedunculos vestitis; calycis laciniis valde inaequalibus late oblongis obtusis 0.8–1.0 cm. longis extus plus minusve ferrugineo-papillatis margine ciliolatis intus in margine uniglandulosis; corollae lobis oblique et late obovato-reniformibus 1.0–1.25 cm. longis paulo reflexis tubo 4.0–4.5 cm. longo usque ⅓ altitudinem anguste cylindrico dein staminigero et sensim cylindrico-conico-dilatato; antheris linearis ca. 0.5 cm. longis dorso minute papillato-striatis; ovariis ovoideis minutissime papillatis nectarium cupulatum apice crenulatum paulo superantibus; folliculis ignotis.—"Peru, Dept. Loreto: Mishuyacu, near Iquitos, alt. 100 m.; forest, Dec., 1929." *G. Klug 657* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Although closely allied to *Odontadenia Cururu* (Mart.) K. Sch., *O. augusta* is distinct because of its much larger leaves, its longer, oblong calyx-lobes, and, particularly, the very shallow, annular nectary which does not conceal the ovary as in the former species.

Odontadenia affinis Woodson, sp. nov., fruticosa volubilis; ramis teretibus longitudinaliter striatis glabris lenticellas parvas paucas gerentibus in sicco rubidulo-coloratis; foliis oppositis breviter petiolatis subcoriaceis oblongo-ovatis apice breviter et obtuse cuspidatis basi acutiusculis 6–8 cm. longis 3.5–4.0 cm. latis omnino glabris supra fuscentibus subtus olivaceo-viridibus venis transversis prominulis petiolo 0.5–0.75 cm. longo in annulo obscuro stipularum cincto; paniculis lateralibus paucifloris breviuscule (3–4 cm.) pedunculatis; bracteis squamosis minimis; pedicellis glabris ca. 0.5 cm. longis; calycis laciniis ovatis obtusiusculis plus minusve conspicue inaequalibus 0.4–0.5 cm. longis extus glabris vel margine minutissime ciliolatis intus in margine

positis 3–4 glandulis; corollae lobis oblique obovatis 1.5–2.0 cm. longis paulo reflexis tubo 3.5–4.0 cm. longo usque ½ altitudinem anguste cylindrico dein sensim longiuscule conico-dilatato dimidiam altitudinem partis angustae staminigero; antheris anguste lanceolatis ca. 0.5 cm. longis dorso minutissime papillatis; ovariis breviter ovoideis glabris nectarium cupulatum crenulatum ca. bis terve superantibus; folliculis ignotis.—"Peru, Dept. Loreto: Balsapuerto (lower Rio Huallaga basin); alt. 150–350 m.; dense forest, Aug. 28–30, 1929." *E. P. Killip & A. C. Smith 28609* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

The insertion of the stamens midway within the narrowly cylindrical corolla-throat may be interpreted as indicating a close relationship of *Odontadenia affinis* with *O. cognata*. From the latter species, however, *O. affinis* differs because of the smaller leaves and calyx-lobes, the lateral, few-flowered inflorescence, the proportionally longer corolla-tube, and the glabrous ovary about two or three times surpassing the altitude of the shallow, annular nectary.

Odontadenia glauca Woodson, sp. nov., fruticosa volubilis; ramis teretibus evidenter gracilibus longitudinaliter striatis lenticellas parvas paucas gerentibus; foliis oppositis breviter petiolatis coriaceis in sicco margine revolutis oblongo-obovatis apice breviter et acute acuminatis basi rotundatis 6.5–8.0 cm. longis, 3.5–4.5 cm. latis supra viridibus nitidis subtus glaucis venulis transversis distinctissimis petiolo 0.3–0.5 cm. longo superne breviore; paniculis subterminalibus (vel lateralibus ?) paucifloris pedunculo brevissimi petiolis subaequante; bracteis squamosis minimis; pedicellis ca. 1 cm. longis gracilibus glabris; calycis laciniis subaequalibus ovato-triangularibus acutiusculis ca. 0.1 cm. longis vix imbricatis glabris intus in margine uniglandulosis; corollae lobis oblique oblongis in alabastro 1.0–1.25 cm. longis tubo 1.5–2.0 cm. longo gracili usque ½ altitudinem cylindrico dein staminigero et anguste tubuloso-dilatato; antheris oblongo-lanceolatis acutis ca. 0.6 cm. longis dorso glabris; ovariis ovoideis glabris nectarium 5-lobatum paulo superantibus; folliculis ignotis.—"Venezuela, Amazonas Territory: Cerro Yapacana, upper Rio Orinoco; alt.

about 100 m., April, 1931." *E. G. Holt & E. R. Blake 750* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

One of the most conspicuous and natural groups of species within the genus *Odontadenia* is that which centers about *O. nitida* (Vahl) Muell.-Arg., and comprises, in addition to that species, *O. hypoglauca*, *O. coriacea*, *O. geminata*, and *O. polyneura*. To this group *O. glauca* must be added, since it possesses the shining, glaucous foliage, the narrow corolla-tube, and the barely concrescent nectaries common to all. From all the species enumerated, however, it differs because of the smaller flowers, the extremely small (0.1 cm.) lobes of the calyx, which can scarcely be described as sheathing as in all other species of the genus, and the strongly revolute margin of the leaves.

Malouetia Killipii Woodson, sp. nov., arborea erecta ca. 10–12 m. alta; ramulis gracilibus teretibus in sicco longitudinaliter striatis lenticellas parvas et remotas gerentibus; foliis oppositis brevissime petiolatis membranaceis vel subcoriaceis late ovato-lanceolatis apice longe et obtuse acuminatis basi subiter attenuatis acutisque cum petiolo ca. 0.25 cm. longo 20–25 cm. longis 6–8 cm. latis supra glabris subtus sparse et tenuissime puberulis; cymis lateralibus vel terminalibus umbellatis brevissime pedunculatis ca. 10–20-floris; pedicellis glabris 0.75–1.0 cm. longis; bracteis squamosis minimis; calycis lobis ovato-reniformibus obtusiusculis 0.1 cm. longis 0.15–0.2 cm. latis patente inaequalibus extus apice minutissime puberulo-papillatis intus in marginibus uniglandulosis; corollae lobis ovatis acutisque 0.75 cm. longis 0.5 cm. latis extus glabris intus dense puberulo-papillatis valde reflexis tubo cylindrico basi paulo dilatato ca. 1 cm. longo saepissime glabro fauce obscure 5-squamato; antheris exsertis dorso minute et dense papillatis; nectario e glandulis 5 ovoideis truncatis subliberis ovario dimidio aequante; folliculis ignotis.—"Peru, Dept. Loreto: Iquitos, woods, alt. about 100 m., Sept. 26, 1929." *E. P. Killip & A. C. Smith 29860* (Mo. Bot. Garden Herbarium, TYPE, U. S. National Herbarium, duplicate).

Until the discovery of the immediately preceding species, there had been but one *Malouetia* reported as possessing a foliar

indument. Both species are natives of the upper Amazon valley. The previously published species, *M. pubescens* Mgf., however, is apparently to be found in a slightly different territory than that of *M. Killipii*, namely the upper Rio Branco, near S. Marcos. *M. Killipii* differs from *M. pubescens* in several important particulars. In the former the leaves are ovate-lanceolate, glabrous above, sparsely and minutely puberulent beneath, the inflorescence relatively many-flowered, the pedicels 0.75–1.0 cm. long and absolutely glabrous, the calyx-lobes ovate-reniform (about twice as broad as long), the corolla-tube 1 cm. long and the lobes but 0.75 cm. long, and the anthers merely papillate dorsally. On the other hand, *M. pubescens* is described (Mgf. Notizblatt **9**: 88. 1924) as having ovate leaves which are sparsely pilose above and very densely velutinous beneath, a few-flowered inflorescence, ovate calyx-lobes (about twice as long as broad), pubescent pedicels only 0.5 cm. long, the corolla-tube 0.3 cm. long and the lobes 0.6 cm. long, and anthers which are densely hirsute dorsally.

Macropharynx spectabilis (Stadelm.) Woodson, n. comb.
Echites spectabilis Stadelm. Flora **24**: I Beibl. 44. 1841.
Elytropus spectabilis (Stadelm.) Miers, Apoc. S. Am. 116. 1878.
Macropharynx fistulosa Rusby, Mem. N. Y. Bot. Gard. **7**: 329. *t. 6*. 1927.

Prestonia agglutinata (Jacq.) Woodson, n. comb.
Echites agglutinata Jacq. Enum. Pl. Carib. 13. 1760.
Echites circinalis Sw. Prodr. 52. 1788.
Haemadictyon circinalis (Sw.) G. Don, Dict. **4**: 83. 1838.
Anechites adglutinata (Jacq.) Miers, Apoc. S. Am. 236. 1878.

Prestonia Dusenii (Malme) Woodson, n. comb.
Echites Dusenii Malme, Arkiv f. Bot. **22A²**: 9. 1928.

Prestonia coalita (Vell.) Woodson, n. comb.
Echites coalita Vell. Fl. Flum. 112. 1830; Icon. **3**: *t. 40*. 1827.
Rhaptocarpus coalitus (Vell.) Miers, *l. c.* 152. 1878.

The three species enumerated above, with the possible addition of a very few others whose specific validity has not been fully

established as yet, constitute a small and very natural group the generic identity of which has been brought into dispute upon several occasions. From *Echites* (*sensu strictiore*), the group of species enumerated differs in the inflorescence, which is racemose, and in the thickened annulus of the corolla orifice. *Anechites*, on the other hand, is a genus of an entirely different subfamily, namely, Plumeroideae, which can include the foregoing species under no circumstances. *Rhaptocarpus* is a genus of no morphological validity, especially founded by Miers for the inclusion of *P. coalita*.

All three species display the essential characteristics of *Prestonia*, which may be epitomized as follows: anthers bearing two parallel sporangia ventrally upon an enlarged, sterile, basally 2-pronged connective; clavuncle fusiform; calyx-lobes bearing a solitary, internal, glandular appendage; orifice of the corolla-throat constricted by a thickened annulus; leaves eglandular. The species do not possess the five internal, strap-shaped appendages attached to the corolla-tube just above the insertion of the stamens, it is true, but those appendages should not be considered with undue emphasis, as they may or may not occur among species of indubitable congenericity (cf. *P. Muelleri* Rusby and *P. Riedelii* (Muell.-Arg.) Mgf.).

It is significant that G. Don transferred *E. circinalis* Sw. to *Haemadictyon* Lindl., a genus which is almost universally considered to be synonymous with *Prestonia* R. Br. at the present time. It is interesting to find, furthermore, that Miers also recognized the affinity of the species included under his genus *Rhaptocarpus* with those of *Prestonia* (Miers, Apoc. S. Am. 151. 1878), but mistook the undeveloped fruit of the only specimen of the former which he was able to examine for a bilocular, syncarpous capsule instead of two confluent follicles in a very immature state (to the fancied resemblance of which he frankly coined the generic name!).

Prestonia portobellensis (Beurl.) Woodson, n. comb.

Echites portobellensis Beurl. Vet. Akad. Handl. Stockh. 137. 1854 (1856).

Prestonia (*Haemadictyon*) *macrocarpa* Hemsl. Biol. Cent.-Am. Bot. **2**: 311. 1881.

The material upon which Beurling based his species is represented by two specimens collected by Billberg in April, 1826, at Porto Bello, Province of Colon, Panama, "*in silvis ad littora.*" These two specimens are in an excellent state of preservation at the present time, and are deposited in the Botanical Museum at Stockholm. *Fendler 250*, cited by Hemsley as a cotype of *P. macrocarpa*, is represented by a duplicate in the herbarium of the Missouri Botanical Garden, and has been found by the writer to be identical with the specimens of Billberg. Since *Fendler 250* bears the data "Chagres, Isthmus of Panama," it is clear that the two collections were made in the same general locality. The species is apparently frequent from Guatemala to Colombia.

Prestonia velutina Woodson, sp. nov., suffruticosa volubilis pauce ramosa; ramis gracilibus flexuosis junioribus dense luteo-puberulis; foliis oppositis brevissime petiolatis membranaceis elliptico-lanceolatis apice acuminatis basi paulo attenuatis et saepissime obtusiusculis cum petiolo 5–8 cm. longis 1.5–2.5 cm. latis supra sparse puberulo-hirtellis subtus tenue luteo-velutinis; racemis axillaribus alternatis 10–15-floris pedunculo ca. 4 cm. longo breviter luteo-hirtello; bracteis subfoliaceis oblongo-ovatis parce hirtellis 0.2–0.3 cm. longis pedicellis 2–3-plo brevioribus; lobis calycis ovato-lanceolatis acuminatis subfoliaceis ca. 0.75 cm. longis extus sparse et tenue luteo-hirtellis intus glabris basi uniglandulosis; corollae lobis oblique obovatis dolabriformibus 0.5–0.75 cm. longis valde reflexis tubo anguste cylindrico 1.0–1.5 cm. longo fauce staminigero squamis linearibus exsertis ca. 0.5 cm. longis; nectario 5-lobo ca. 0.15 cm. alto ovariis oblongoideis glabris subaequante; folliculis ignotis.—"Colombia: Hondo, Aug., 1919." *Bro. Ariste-Joseph s. n.* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Prestonia velutina simulates *P. acutifolia* (Benth.) K. Sch. in the general outline of the leaves and size and disposition of the flowers. In the former, however, the calyx-lobes are much more conspicuous and subfoliaceous, and the whole plant, with the exception of the exterior of the corolla, is covered with a fine, yellowish, velutinous or hirtellous indument.

Prestonia isthmica Woodson, sp. nov., fruticosa; ramis volubilibus teretibus sat crassis longitudinaliter striatis dense luteo-hirtellis; foliis oppositis brevissime petiolatis membranaceis oblongo-obovatis apice breviter et acute acuminatis basi obtusis et obscure auriculatis 15–20 cm. longis 10–13 cm. latis supra minute et sparse strigillosis mox glabratis subtus leviter luteo-hirtellis petiolo ca. 0.3 cm. longo ut in ramis vestito; racemis bostrycino-umbelliformibus lateralibus alternatis 4–6-floris pallide luteo-hirtellis pedunculo ca. 3 cm. longo; bracteis subfoliaceis ovato-lanceolatis 1.0–1.5 cm. longis; pedicellis 0.5–0.75 cm. longis; lobis calycis ovato-lanceolatis apice longe acuminatis basi cordatis 1.5–2.0 cm. longis 0.3–0.5 cm. latis intus basi uniglandulosis; corollae tubo longe cyclindrico 3.0–3.5 cm. longo basi ca. 0.3 cm. diametro fauce staminigero appendiculato-constricto extus pallide sericeo intus hirtello haud squamuligero lobis oblique obovatis ca. 1.5 cm. longis extus intusque glabris valde reflexis; nectario e glandulis subliberis oblongoideis 0.4–0.5 cm. longis ovaria ovoidea glabra bis terve superantibus; folliculis ignotis.—"Costa Rica: between Aserri and Tarbaca, Prov. San Jose, alt. 1200–1700 m., Dec. 6, 1925." *P. C. Standley 41332* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Prestonia isthmica differs from the neighboring *P. mexicana* A. DC. because of its longer and narrower corolla-tube with glabrous lobes, its long-attenuate, cordate calyx-segments, and its broader and larger leaves. The technical distinction of greatest significance is found in the nectaries, which are ovoid-quadrate, concrescent, and barely attain the length of the carpels in the latter species, and are narrowly oblongoid, essentially separate, and about twice surpass the length of the carpels in the former.

Laubertia Sanctae-Martae (Rusby) Woodson, n. comb.
Echites Sanctae-Martae Rusby, Descr. S. Am. Pl. 85. 1920.

Laubertia Pringlei (Greenm.) Woodson, n. comb.
Streptotrachelus Pringlei Greenm. Proc. Am. Acad. **32**: 298. 1897.
Prestonia Langlassei Standl. Contr. U. S. Nat. Herb. **23**: 1159. 1924.

The genus *Laubertia*, established by A. de Candolle in 1844 with a single species, *L. Boissieri*, until recently has been perhaps the outstanding enigma of the Apocynaceae. The type species was based upon two specimens collected by Pavon in Peru and which are now deposited in the Herbier Boissier at the University of Geneva. Apparently these are the only representatives of the original collection in existence at the present time. Although adequately described by de Candolle, the genus immediately fell into disuse, probably because few subsequent collectors retraced the itinerary of Pavon until recently.

Mueller-Argoviensis did not mention the genus in any work which the present writer has been able to consult. Miers was fortunately content merely to refer to the genus in his monograph 'On the Apocynaceae of South America,' and to call Grisebach to account for using *Laubertia* as a sectional designation under *Echites* to include three species of the West Indies properly referable to *Rhabdadenia*. K. Schumann, in Engler & Prantl's 'Natürlichen Pflanzenfamilien,' was evidently without first-hand knowledge concerning *Laubertia*, placing it between the distantly related genera *Rhabdadenia* and *Mandevilla*, and keying it upon the character of a three-lobed "discus," although later correctly describing that structure as five-lobed.

Laubertia is one of the most distinct and natural genera of the subfamily Echitoideae of Apocynaceae. As in *Prestonia*, the orifice of the corolla is conspicuously thickened and the tips of the anthers are slightly exserted, but unlike that of the latter genus, the calyx is eglandular. At present, the genus consists of only three species: *L. Boissieri* in Peru, *L. Sanctae-Martae* in Colombia, and *L. Pringlei* in southern Mexico.

NEW SOUTH AMERICAN ASCLEPIADACEAE

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Blepharodon minimus Woodson, sp. nov., herbacea erecta plus minusve diffusa ca. 2 dm. alta; caulibus filiformibus teretibus laxe foliatis glabris vel junioribus tenuissime puberulis; foliis patentibus patulisve oppositis brevissime petiolatis anguste linearibus 3–6 cm. longis 0.1–0.2 cm. latis utrinque glabris margine tenue ciliolatis in sicco revolutis petiolo glabro ca. 0.1 cm. longo; inflorescentiis axillaribus alternatis umbelliformibus 2–3-floris omnino glabris pedunculo ca. 0.1 cm. longo; pedicellis 0.2–0.3 cm. longis; calycis lobis ovatis acutiusculis ca. 0.1 cm. longis omnino glabris basi intus in marginibus tectis 2–3-glandulosis; corollae rotatae ostio 0.1–0.2 cm. diametro lobis ovatis acutis omnino glabris ca. 0.3 cm. longis; gynostegio sessili late conico ca. 0.1 cm. alto; coronae foliolis cuculattis late oblongo-ovatis gynostegio subaequantibus; antheris trapezoideo-oblongis appendice hyalina oblonga obtusa; polliniis oblique obovoideis; caudiculis pendentibus mediocribus; retinaculo anguste rhomboideo-oblongoideo polliniis paulo minore; folliculis ignotis.—"Colombia, Dept. Tolima: 'El Convenio,' west of San Lorenzo. Open hilltop, alt. 1000–1200 m., Dec. 29–30, 1917." *F. W. Pennell 3487* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Blepharodon minimus is most closely allied to *B. suberectus* Schltr., from which it differs in having much smaller, nearly sessile leaves with ciliolate margins, a much-reduced inflorescence with extremely short peduncle and pedicels, and an entirely glabrous calyx. The two species also differ in more technical details, such as the shape and size of the corona segments, which are oblong-acuminate and slightly surpass the gynostegium in *B. suberectus*, and are broadly ovate-oblong, obtuse, and somewhat shorter than the gynostegium in *B. minimus*. An additional detail of significance in the reproductive organs is found in the shape of the retinaculum, which is ovoid in the former species and narrowly rhomboid-oblongoid in the latter.

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Stephanotella Killipii Woodson, sp. nov., suffruticosa; ramis volubilibus teretibus junioribus tenuissime et sparse puberulis maturioribus glabratis; foliis oppositis petiolatis membranaceis ovato-ellipticis apice breviter et obtuse acuminatis basi obtusis 10–20 cm. longis 6–11 cm. latis supra glabris subtus tenuissime et sparse puberulis petiolo 1.5–2.0 cm. longo tenuissime puberulo in annulo obscuro stipularum instructo; cymis axillaribus alternatis 2–3-chotomis subumbelliformibus 10–20-floris pedunculo petiolos aequante vel paulo superante; bracteis scariaceis ovatis minimis; pedicellis ca. 0.5 cm. longis; calycis segmentis scariaceis oblongo-ovatis obtusiusculis 0.3 cm. longis tenuissime puberulis basi intus in marginibus uniglandulosis; corollae tubo cylindrico 0.5 cm. longo basi paulo dilatato lobis oblique ovato-oblongis 0.4–0.5 cm. longis margine tenue ciliolatis; gynostegio stipitati ca. 0.2 cm. alto obtuse rostrato; coronae foliolis connatis gynostegio adnatis et paulo breviore; antheris elongatis membrana hyalina obtusa terminatis; polliniis oblique obovoideis erectis; caudiculis mediocribus; retinaculo anguste ligulato polliniis paulo breviore; folliculis ignotis.—"Peru, Dept. Loreto: wooded banks of Rio Itaya, above Iquitos, alt. about 110 m., Sept. 17–22, 1929." *E. P. Killip & A. C. Smith 29392* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, duplicate and analytical drawings).

The genus *Stephanotella* was established in 1885 by Fournier with a single species, *S. Glaziovii*, based upon a specimen collected by Glaziou in the neighborhood of Rio de Janeiro. Fortunately, the plant was well figured and described by Fournier, for it is evidently extremely rare, as no additional species have been ascribed to the genus until the present and no specimens of the original species are to be found in any of the larger American herbaria. It is noteworthy, therefore, that a second species of the genus should have been encountered by Messrs. Killip & Smith upon the Rio Itaya, a Peruvian tributary of the upper Amazon.

A comparison of the original description and illustration of *Stephanotella Glaziovii* (Fourn. in Martius, Fl. Bras. 6[4]: 326–327. *t. 96*. 1885) with the specimen collected by Killip & Smith reveals that the two are quite similar in general appearance, but

differ in both superficial and technical characteristics. The leaves of *S. Glaziovii* are described and figured as ovate-cordate, with a broad sinus, whereas those of *S. Killipii* are ovate-elliptic, with an obtuse base. Those of the former species, moreover, are described as "pilose." whereas those of the latter are always glabrous above, with a sparse and minute puberulous indument upon the lower surface of young individuals only. The leaves of the latter species, moreover, are evidently about twice the size of those of the former.

More technical differences between the two species are abundant and of almost generic importance. The interior calycine emergences or "squamellae" of *S. Killipii* are extremely small and occur separately in the axils of the calyx-lobes, but the calyx of *S. Glaziovii* is described emphatically as "*non solum in axillis sed inter sepala et corollam pluriglandulosis.*" The lobes of the corona in *S. Killipii* are completely connate and are somewhat surpassed by the rostrum of the gynostegium. The corona of *S. Glaziovii*, on the other hand, is deeply 5-cleft almost to the base, and the narrow appendices of the anthers conspicuously exceed the gynostegium. The retinaculum of either species, finally, is very distinct, that of *S. Glaziovii* being ovoid and very thick, whereas that of *S. Killipii* is merely an attenuate ligule.

Macroscepis equatorialis Woodson, sp. nov., suffruticosa; ramis volubilibus teretibus in sicco longitudinaliter striatis dense luteopilosis pilis dissimilibus tum brevibus simplicibus tum multo longioribus multicellularibus sicut ad petiolos pedunculos pedicellosque; foliis oppositis petiolatis membranaceis obovatis apice breviter et obtuse cuspidatis basi anguste cordatis 15–20 cm. longis 13–15 cm. latis supra sparse strigosis subtus farinulentis et longe pilosis petiolo 4–5 cm. longo in annulo obscuro stipularum instructo; inflorescentiis axillaribus alternatis umbelliformibus 6–8-floris pedunculo ca. 1 cm. longo; bracteis linearibus ca. 1.5 cm. longis viridibus dense pilosis; pedicellis ca. 0.5 cm. longis; calycis lobis scariaceis late ovatis acutis 1 cm. longis 0.75 cm. latis brevissime puberulis apice longe pilosis basi intus in marginibus uniglandulosis; corollae tubo cylindrico-campanulato 0.75 cm. longo fauce constricto et parce appendiculato ca. 0.5 cm.

diametro lobis ovatis acutiusculis 0.75 cm. longis 0.5 cm. latis extus intusque brevissime puberulis; gynostegio subsessili; coronae foliolis corollae tubo fere ad faucem et tubo stamineo adnatis omnino inclusis apice introrsum replicatis; polliniis oblongo-obovoideis pendulis; caudiculis brevioribus apice dilatatis; retinaculo oblongo leviter compresso apice rotundato basi acutiusculo polliniis multo breviore; stigmate 5-lobo in medio excavato; folliculis solitariis ovoideis basi rotundatis apice acuminatis usque ad 9 cm. longis ad 3.5 cm. crassis late 5-alatis alis ca. 0.5 cm. latis laevibus glabris.—"Ecuador, Prov. Guayas: Oil Camp between Guayaquil and Salinas, alt. 0–100 m., June 21–24, 1923." *A. S. Hitchcock 20109* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

M. equatorialis is probably most closely related to *M. barbata* S. F. Blake, from which it differs in having somewhat larger leaves of a different shape and indument, a calyx which is definitely glandular within, and a shorter corolla-tube with lobes which are not emarginate as in the latter species. In addition, the coloration of the flowers is very probably different, that of *M. equatorialis* being described as "brown" and that of *M. barbata* as "greenish . . . the lobes dark green with a narrow pale margin" by the respective collectors of the type specimens of either species.

Phaeostemma tigrina Woodson, sp. nov., suffruticosa; ramis volubilibus teretibus dense luteo-hirtellis sicut ad petiolos pedunculos et pedicellos; foliis oppositis petiolatis membranaceis ovato-cordatis apice breviter et acute acuminatis basi late auriculatis 10–12 cm. longis 7–8 cm. latis supra densissime bullato-strigillosis subtus luteo-hirtellis petiolo 3.0–3.5 cm. longo in annulo obscuro stipularum instructo; inflorescentiis axillaribus alternatis corymboso-umbelliformibus 8–10-floris pedunculo 9–10 cm. longo; bracteis scariaceis minimis; calycis lobis linearibus obtusiusculis ca. 0.5 cm. longis ca. 0.1 cm. latis luteo-hirtellis basi intus in marginibus uniglandulosis glandulis linearibus minute pilosis; corollae rotatae pulchre flavo-fulvo-reticulatae tubo breviter cylindrico-campanulato 0.3–0.4 cm. longo glabro fauce ca. 0.5 cm. diametro lobis ovatis acutis 0.7–0.8 cm. longis

0.4–0.5 cm. latis extus minute puberulo-papillatis intus glabriusculis; gynostegio subsessili; coronae foliolis tubo corollae fere aequantibus interioribus connatis gynostegio et tubo stamineo adnatis exterioribus obtuse bilobatis inferius in medio carinatis; polliniis anguste oblongo-obovoideis pendulis; caudiculis horizontalibus auriculatis; retinaculo sagittato leviter compresso apice acutiusculo basi subhastato; stigmate depresso 5-lobo ca. 0.4 cm. diametro; folliculis ignotis.—"Colombia, Dept. El Cauca: 'Caliguala,' Coconuco, cliff near Rio San Andreas, alt. 2700–3000 m., June 14–18, 1922." *F. W. Pennell 7151* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

Ph. grandifolia Rusby, the only species of *Phaeostemma* previously reported from Colombia, should probably be regarded as the nearest ally of the foregoing. From *Ph. grandifolia*, *Ph. tigrina* differs in having much smaller leaves with a different indument and with much shorter petioles, shorter and much narrower calyx-lobes, and a smaller corolla of somewhat different construction. In addition, technical differences in the reproductive organs exist. The coloration of the corolla of *Ph. grandifolia* is reported as "purple-veined and finely white-spotted, corona purple" (Rusby), and that of *Ph. tigrina* as "cossack-green, veiny on cream petals, cream center" (Pennell). The "cream center" cited by Dr. Pennell is evidently equivalent to the "corona" of Dr. Rusby's plant, and both probably refer to the plane, pentagonal stigma.

Exolobus marmoreus Woodson, sp. nov., suffruticosa alte scandens; ramis ramulisque volubilibus in sicco longitudinaliter striatis laxe foliatis dense luteo-hirtellis sicut ad petiolos pedunculos et pedicellos; foliis oppositis petiolatis membranaceis ovato-oblongis apice acute acuminatis basi late cordatis 5–9 cm. longis 3.5–6.0 cm. latis supra dense strigillosis subtus molliter luteo-puberulis petiolo 2.0–2.5 cm. longo in annulo obscuro stipularum instructo; cymis corymbiformibus axillaribus alternatisque 10–15-floris pedunculo 2–3 cm. longo; pedicellis 2.0–2.5 cm. longis; calycis segmentis lanceolatis acutiusculis ca. 0.7 cm. longis 0.1–0.2 cm. latis extus laxe pilosulis intus glabris

basi in marginibus uniglandulosis; corollae rotatae lobis ovato-lanceolatis acutiusculis usque ad 1 cm. longis 0.3–0.4 cm. latis pulchre virido-marmoreis extus glabris intus papillatis basi leviter puberulis; coronae exterioris annularis depressae leviter 5-lobatae lobis minutissime puberulis; coronae interioris foliolis oblongo-spathulatis gynostegio et tubo stamineo adnatis; antherarum angulis superioribus anguste reniformibus divergentibus; polliniis oblique pyriformibus pendulis; caudiculis horizontalibus auriculatis perbrevibus; retinaculo minuto-rhomboideo polliniis fere 6-plo breviore; folliculis ignotis.—"Colombia, Dept. Norte de Santander: between Pamplonita and Chinacota, Rio Pamplonita Valley, alt. 1300–1800 m., March 17, 1927, *E. P. Killip & A. C. Smith 20748* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

From *E. patens* (Dcne.) Fourn., which is evidently the most widespread species of *Exolobus* in South America, *E. marmoreus* differs superficially by reason of its broader leaves with shorter petioles, its more floribund inflorescence, and its smaller corolla with proportionally longer calyx-lobes. Several technical differences occur in the reproductive organs, the most conspicuous of which is the shape of the anther appendages, which are obovate in *E. patens* and narrowly reniform in *E. marmoreus*. The specific name refers to the dark greenish reticulation of the cream-colored corolla-lobes.

Marsdenia lauretiana Woodson, sp. nov., suffruticosa volubilis omnino glabra; ramis teretibus sat crassis longitudinaliter striatis; foliis oppositis petiolatis subcoriaceis elliptico-obovatis apice breviter et acute acuminatis basi attenuatis acutisque 7–10 cm. longis 2.0–4.5 cm. latis petiolo ca. 1 cm. longo in annulo obscuro glandulo-appendiculato instructo; cymis lateralibus alternatis umbelliformibus 6–10-floris pedunculo ca. 0.5 cm. longo; bracteis scariaceis vix apertis; pedicellis pedunculos aequantibus vel paulo superantibus; calycis laciniis late ovato-deltoideis obtusissimis ca. 0.3 cm. longis 0.3–0.4 cm. latis extus glabris vel obscurissime papillatis intus glabris in marginibus 3–4-glandulosis margine ciliolatis; corollae carnosae plus minusve maculatae tubo breviter cylindrico fauce constricto ca. 0.4 cm. longo

basi ca. 0.3 cm. diametro extus minute et sparse papillato intus in parte infra alas antherarum sita hirtello lobis patentibus late obovatis apice rotundatis ca. 0.3 cm. longis margine ciliolatis; gynostegio breviter stipitato; filamentis staminalibus brevibus alis tenuibus membranis antherarum apice obtusis; coronae foliolis dorso staminibus adnatis basi volvatis supra acumine lato ornatis antherarum membranis dimidia breviore; polliniis obovoideis erectis; caudiculis vix brevioribus primum descendentibus dein horizontalibus; retinaculo late elliptico superiore parte subacuminato polliniis multo breviore; stigmatis rostro conoideo muriculato apice obtuso antherarum membranas paulo superante; folliculis ignotis.—"Peru, Dept. Loreto: Mishuyacu, near Iquitos, alt. 100 m., forest, Oct.–Nov., 1929." *G. Klug 477* (U. S. National Herbarium, TYPE, Mo. Bot. Garden Herbarium, photograph and analytical drawings).

When referred to the identificatory keys of Rothe's[1] revision of the genus *Marsdenia*, *M. lauretiana* is readily included within the section *Ruehssia* subsection *Mollissimae*. The species is evidently most closely related to *M. mollissima* Fourn., but strongly contrasts with it because of the complete glabrity of the vegetative parts. The leaves of *M. lauretiana*, furthermore, are elliptic-obovate and subcoriaceous, whereas those of *M. mollissima* are ovate-cordate and membranaceous. Although sufficiently similar to include them within the same subsection, the reproductive organs also differ markedly.

[1] Rothe, in Engl. Bot. Jahrb. 52: 354–434. 1915.

SOME EFFECTS OF ULTRA-VIOLET RADIATION UPON THE CALCIUM AND PHOSPHORUS CONTENT OF HIGHER PLANTS

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I. REVIEW OF PREVIOUS WORK AND STATEMENT OF THE PROBLEM

That ultra-violet radiation exerts an accelerative effect upon calcium and phosphorus metabolism, especially the former, has been demonstrated repeatedly. Among the numerous workers who have made studies in the field are these: Steenbock and Nelson ('23), who showed that ultra-violet rays restore growth in rats deprived of fat-soluble vitamines; Orr, Holt, Wilkins, and Boone ('23), who demonstrated that ultra-violet rays cause large amounts of calcium and phosphorus to be retained in the body; Vignard, Mouriquand, Chassard and Bernheim ('23), who brought forth radiographic evidence that ultra-violet promotes the precipitation of calcium at the junctures of bone and cartilage; Clark ('23), who showed that the diffusible calcium of the blood is higher after the serum is exposed to ultra-violet radiation; Grant and Gates ('24), who found that the blood calcium of irradiated rabbits increases considerably over that of controls. Other workers in the field have been Huldschinsky, Hess, Powers, Funk, and Park ('23), the latter of whom gives an inclusive review of the literature concerning the effects of radiant energy on rickets.

The effects of a similar treatment with ultra-violet radiation upon the calcium and phosphorus content of higher plants seemed to the authors to constitute a problem in comparative physiology worthy of investigation, because thereby an additional contribution might be made to the long list of physiologic analogies between plants and animals.

In a survey of the literature concerning the effect of ultra-violet radiation upon plants, evidence of previous work upon this question has been almost entirely lacking. Indeed it seems that Beeskow ('27) has been the only investigator to report upon the effects of ultra-violet rays on the calcium and phosphorus of plants. His work, mentioned briefly at Nashville, appeared to show that rayed plants of *Zea Mays* exhibited increased calcium and phosphorus content. In the present paper the authors have attempted to present additional and more complete data concerning this particular aspect of ultra-violet physiology.

II. MATERIAL

The plant material used in this work consisted of tomatoes and cucumbers, as employed in a previous work (Fuller, '31) on the stimulatory effects of radiation from a quartz mercury vapor arc. In that work plants were rayed according to various schedules with different screens. Of these groups, one, rayed daily for five weeks with Vita-glass at one hundred inches from the arc, showed definitely accelerated growth as compared with the controls, which received no ultra-violet radiation; the rayed and control sets were designated respectively as E and A. In comparison with the controls the plants rayed under Vita-glass were nearly one-third taller at the end of the experiment, showed a slightly increased dry weight and ash content, and were in every respect extremely healthy. Dry powders of these plants were used for the analytic work which is described below.

III. METHODS

Because of the varying analytical results obtained from different procedures, the methods used in this work are described in detail, even at the risk of repeating information already present in chemical literature.

Preparation of sample.—One-gram portions of the powdered, air-dried sample were thoroughly ashed by a Fischer burner and the residue dissolved in the crucible with 2 cc. of concentrated hydrochloric acid. The contents of the crucible were then washed into a porcelain casserole with about 50 cc. of dilute hydrochloric acid (1 : 3) and evaporated to dryness on the water bath; the

residue was baked two hours in an electric oven at 120° C. to render the silica insoluble. To the residue was then added 150 cc. of dilute hydrochloric acid, after which the casserole was allowed to stand on the water bath half an hour to insure the complete dissolving of the soluble constituents. Silica was removed by filtering through a hard filter-paper. The entire silica-free filtrate prepared from each gram of air-dried sample was used in a single determination of calcium or phosphorus in order to avoid the labor of preparing exact volumetric aliquots of a single prepared solution.

Estimation of calcium.—Calcium was estimated by titration with .1 N potassium permanganate according to the method of McCrudden ('09) and Mitchell ('21). Previous experience of the authors has shown this method to be susceptible of extraordinary accuracy, the limit of which is determined largely by the accuracy with which the original samples are taken. The prepared solution from one gram of air-dried sample was transferred to a 300-cc. beaker and made up to a volume of about 200 cc. Two drops of methyl orange were added and the solution made slightly alkaline with ammonium hydroxide (1 : 1). Dilute hydrochloric acid was then added drop by drop with constant stirring until the indicator showed a faintly acid reaction. Then 10 cc. of .5 N hydrochloric acid and 10 cc. of a 2.5 per cent solution of oxalic acid were added. The mixture was boiled, and 20 cc. of a saturated solution of ammonium oxalate added slowly with constant stirring. The mixture was heated until the precipitate became sufficiently granular for filtration, then cooled, and 8 cc. of a 20 per cent solution of sodium acetate (or enough to bring the solution to an alkaline reaction) were added. After standing over night the calcium oxalate was removed by filtration and washed with hot water until free from chlorides. The filter-paper was ruptured with a stirring rod, and the residue washed with hot water into the original beaker in which the calcium oxalate was precipitated. The precipitate was dissolved by the addition of 10 cc. of sulphuric acid (1 : 1) to the hot mixture. The hot solution was titrated immediately with .1 N potassium permanganate.

Estimation of phosphorus.—Phosphorus was estimated by precipitating with molybdate and weighing as magnesium pyrophos-

phate as described in "Official and Tentative Methods of Analysis" of the Association of Official Agricultural Chemists ('24). The prepared solution from one gram of air-dried sample was made up to about 50 cc. volume with distilled water. Concentrated ammonium hydroxide was added drop by drop with constant stirring until a slight precipitate was formed. This precipitate was dissolved by a few drops of concentrated nitric acid. Since hydrochloric acid had been used as a solvent for the ash, about 15 grams of dry ammonium nitrate were added. The solution was heated, and 40 cc. of molybdate solution were added. The mixture was digested an hour on the water bath, filtered, and the residue washed with dilute ammonium nitrate. The precipitate was dissolved on the filter-paper with ammonium hydroxide (1 : 1) and the paper washed with hot water until the volume of solution and washing was about 100 cc.

Hydrochloric acid (1 : 3) was added drop by drop until only a faint odor of ammonia remained, and the solution cooled in the Kelvinator. To the chilled solution 10 cc. of magnesia mixture were added by means of a burette, drop by drop, with vigorous stirring. After 15 minutes 10 cc. of concentrated ammonium hydroxide were added, and after standing over night the precipitate was filtered on an ashless filter-paper and washed free from chlorides. It was then ignited with a Fischer burner to a constant weight of magnesium pyrophosphate.

TABLE I

STIMULATORY EFFECTS AS EXEMPLIFIED BY WEIGHT DATA

Plant	Average wet weight per plant in gms.		Average dry weight per plant in gms.		Average ash % of dry weight	
	Control A	U-V. E	Control A	U-V. E	Control A	U-V. E
Cucumber	11.75	14.16	1.059	1.423	18.02	20.29
Tomato	10.52	15.18	.8489	1.586	16.98	19.15

TABLE II

DATA ON PHOSPHORUS ANALYSES

Plant	1		2		3	4	
	% P_2O_5 of dry weight		Average uptake of P_2O_5 per plant in gms.		Average % increase of P_2O_5 per plant of the rayed plants	Average % decrease of P_2O_5 in dry weight in rayed plants	
	Control A	U–V. E	Control A	U–V. E		Actual decrease (A–E)	Relative decrease $\left(\frac{A-E}{A} \times 100\right)$
Cucumber	1.498±.034	1.385±.043	.0159	.0197	23.89	.113	7.54
Tomato	1.146±.022	.9865±.025	.0097	.0156	60.82	.159	13.87

Explanation of tables.—Column 3 represents the average per cent increase of the total uptake in grams of P_2O_5 per plant. Column 4 represents two phases of the results relative to dry weight. The first, the actual difference, represents the algebraic difference between the controls and the rayed sets; the second, the percentage of this difference.

TABLE III

DATA ON CALCIUM ANALYSES

Plant	1		2		3	4	
	% CaO of dry weight		Average uptake of CaO per plant in grams		Average % increase of CaO per plant of the rayed plants	Average % increase of CaO in dry weight	
	Control A	U–V. E	Control A	U–V. E		Actual increase (E–A)	Relative increase $\left(\frac{E-A}{A} \times 100\right)$
Cucumber	4.239±.048	4.596±.088	.0449	.0654	45.43	.357	8.44
Tomato	2.769±.022	2.824±.012	.0235	.0448	90.64	.055	1.98

IV. DISCUSSION

From the tables it is obvious that, first, the calcium content of the rayed tomato and cucumber plants is greater than that of the unrayed plants, and second, the phosphorus content of the rayed sets is lower than that of the controls. The results concerning calcium, then, support the findings of Beeskow and show an interesting similarity to the physiologic effects of ultra-violet radiation on animal tissue. As to the results of the phosphorus analyses, however, the condition is reversed—the rayed plants show the lower content, a condition contrary to that found by Beeskow and to that obtaining in animal tissue subjected to ultra-violet. The actual phosphorus *uptake* of the rayed plants is larger, however, than that of the controls, as is shown in column 3, table II, since the rayed plants show a greater amount of growth; but the actual percentage of phosphorus in the latter plants is lower than that of the controls.

No attempt is made in this paper to present an explanation of these phenomena concerning calcium and phosphorus, since data requisite to such an explanation, particularly information about phytosterol activity and vitamine potency, are lacking. The paper does, however, emphasize the definite calcium increase.

V. SUMMARY

1. Tomato and cucumber plants which had been stimulated to greater growth by ultra-violet radiation showed a definite increase in calcium content, calculated as percentage of dry weight.

2. The same plants showed a decrease in phosphorus content, determined in the same manner.

3. The analytic procedure is described in detail.

VI. ACKNOWLEDGMENTS

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STUDIES OF THE EFFECTS OF DIFFERENT LENGTHS OF DAY, WITH VARIATIONS IN TEMPERATURE, ON VEGETATIVE GROWTH AND REPRODUCTION IN COTTON[1]

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Historical Review

The work of Garner and Allard ('20) has aroused considerable interest concerning the seasonal behavior of plants. These investigators have laid particular stress upon the relative lengths of day and night as factors which largely determine the vegetative and reproductive growth of plants. Adams ('20) showed further that the number of capsules formed was largely determined by the length of day. Tjebbes and Uphof ('22) showed that increased length of day hastened the germination of seeds of many cultivated plants. Harvey ('22), by using a wide range of plants, found that many cultivated plants, as well as many common weeds, could bear flowers and fruits in much less time under continuous light than with normal daylight, thus producing more than one generation in a single season. Wanser ('22) states that in winter wheat a certain length of day is necessary in order to stimulate the formation of a jointed stem following the winter rosette condition, and a still longer day for the production of flowers and fruits. Redington ('30) grew a large variety of plants under artificial light, but too few plants of each species to draw reliable conclusions.

Considerable question was raised as to the cause of these effects of light. Koningsberger ('23) suggested that the intensity of light is the effective agency rather than the length of day. Adams ('24) disproved this idea and showed that under the same intensity of light and at the same temperature different species of plants give entirely different results, some flowering with one

[1] An investigation carried out in the graduate laboratory of the Henry Shaw School of Botany of Washington University and submitted as a thesis in partial fulfillment of the requirements for the degree of master of science in the Henry Shaw School of Botany of Washington University.

[2] A fellowship established by the American Creosoting Co.

length of day and others with an entirely different light period. Aso and Umejiro ('24), Munerati ('24), and Tinker ('24) confirmed these findings and further showed that the vegetative growth is directly proportional to the length of daylight when the temperature and other factors are equal. Adams ('25) grew a number of cultivated plants under constant illumination of 700 watts with daylight excluded, and found that the castor bean alone produced viable seeds. Three of the other plants produced normal flowers but no fruits. He suggested that temperature might in some cases compensate for reduced light.

Deats ('25) showed that tomatoes produced more flowers and set more fruits under a long day, whereas pepper set more fruits under a short day. With both species the amount and rate of vegetative growth of various parts of the plants were directly proportional to the length of day. Doroshenko ('27) enlarged on this work, showing that different varieties of the same species varied with the length of day. He showed also that in wheat, barley, and flax the decrease in length of day caused reduction in the size of the cells, reduction in size and increase in number of stomata, and an increased development of veins per unit surface area, whereas a further shortening of the length of day reversed these changes.

Johansson ('27) showed that increased intensity of illumination increased the weight of the root systems of plants in the greenhouse, and that prolonged exposure to weak light also increased the weight of the roots. Stems were similarly affected, but only at the exposure of 6 to 8 hours per day did increased light intensity stimulate leaf production. Zimmerman and Hitchcock ('29) found that in six varieties of dahlia the formation of large storage roots was correlated with a short day, and a fibrous root system with a long day. Flowering appeared to be independent of the formation of storage roots in most of the varieties used but correlated in one or two. Nitrates accumulated in the leaves of the plants exposed to the short day, but little if any in those exposed to the long day.

Garner and Allard ('27), in a preliminary report and later ('31) with more complete data, showed that with a total daily illumination of 12 hours, a progressive shortening of the periods

of alternation of light and darkness resulted in a decided decrease in growth of most plants used. The minimum was reached with light-darkness alternations of about one minute, whereas further shortening of the alternations to 15 seconds gave decided improvement in growth. The injurious effects of the intermediate alternations included apparent destruction of chlorophyll, general etiolation, localized dying of leaf tissue, reduction in leaf development and others. The effects on growth were very similar in both short- and long-day plants but flowering was promoted in the long-day plants. Other combinations of length of day were used, such as the darkening of plants for certain hours during the middle of the day.

The amount of growth and nodule development of soy beans was found by Eaton ('31) to correlate with the length of day and the degree of severity of clipping of the leaves. Nodule development was proportional to the percentage of carbohydrates which was greatly reduced by severe clipping or by a short day.

Temperature has been recognized as an important factor in the rate of growth and time of reproduction of plants. Fung ('11) measured the temperature and relative humidity conditions in which he grew cotton plants in both soil and water culture. Although he grew the plants for only a short time, he found that they were very sensitive to differences in temperature and humidity. The thermal death point was found to be about 129° F. with a relative humidity of 88 per cent. The experiment was not carried sufficiently long to make accurate determinations but he found the optimum conditions for vegetative growth for a certain variety of upland cotton to be a temperature of about 90° F. with a relative humidity of 72 per cent, whereas those for sea island and Egyptian varieties were nearer 85° F. and 70 per cent relative humidity.

Garner and Allard ('30) called attention to the fact that certain varieties of soy beans show a seasonal fluctuation in time of flowering which is correlated with seasonal fluctuations in temperature. The length of day appeared to be the determining factor in the time of flowering in different early and late varieties of soy beans, but changes in temperature either hastened or delayed the period of reproduction.

Eaton ('24) grew a number of plants in the open under a fixed period of illumination of 13 hours per day and three temperatures: 50, 65 and 90° F. during the night. He found that peking soy beans produced more than twice as much vegetative growth at low as at high temperature. The plants grown at a high temperature flowered within 21 days as compared with 45 days at low temperature. Cotton (var. durango) died immediately at the low temperature but grew well and produced a good quantity of fruits at the high temperature. The plants kept at 65° during the night grew rapidly, becoming larger than those at high temperature but did not flower until the experiment was closed. The differences in time of flowering in these sets of plants were almost as great as the differences effected by exposing to varied lengths of day.

Gilbert ('26a) grew soy beans, cosmos, salvia, cotton, and buckwheat under a constant day length, varying temperature and humidity. Flowering was retarded in cotton and soy beans by the lower temperature and higher humidity. Cosmos, on the other hand, flowered earlier under the lower temperature and higher humidity, whereas salvia and buckwheat showed no selective reaction to temperature and humidity. In this experiment the light was kept constant, but in the same year ('26b) Gilbert grew *Xanthium pennsylvanicum* under three different day lengths and varied the temperature. The different day lengths were: (1) the normal day of the winter months, from 10 to 13.1 hours light per day, continued in the spring by covering the plants at the end of the 10-hour light period; (2) a slightly longer day of 13.8 to 14.6 hours per day during the spring months; (3) continuous light. The temperature range was from 65 to 95° F. for his high-temperature plants and 42 to 88° F. for his low-temperature plants as given by average weekly minimum and maximum temperatures. As some of these plants were grown in the open (garden), they can hardly be compared with plants grown in the greenhouse. The higher temperature-short day plants showed indications of flowering 12 to 15 days after planting but few fruits were produced. The higher temperature-long day plants flowered 47 days after planting. The low temperature-short day plants produced staminate flower buds after 116 days. The

lower temperature-long day plants in three different sets showed a minimum vegetative growth before flowering of 92 days and a maximum of 197 days in respective sets. These results do not coincide exactly with the results obtained by the author on cotton, but show the importance of temperature variations in the reproduction of plants. Kellerman ('26) in his review of the discovery of photoperiodism states that cold weather may delay growth, thus delaying flowering, and hot weather may hasten it, but if the light period is not suitable weather conditions cannot cause flowering.

Atkinson as early as 1892 made some careful observations in which he noticed that the changing from one extreme to another of weather conditions caused an increased shedding of all forms in cotton. He noticed also that an excess of water as well as drouth would cause shedding. Balls ('12) in a series of experiments confirmed these observations and showed further that a disturbance of the root system or other injury might cause abscission. He found also that the bolls were shed when young more abundantly than after developing to a certain size when little or no shedding was noticed. Lloyd ('16) suggested irrigation as a possible prevention of boll shedding, as he thought it was largely due to too rapid transpiration. Coit and Hodgson ('18) stated that the June drop of navel oranges is due largely to climatic conditions, the transpiration rate being greater than the absorption rate. Thus the older fruits and leaves draw moisture from the young fruits which in turn causes them to fall. They suggested the growing of such crops as alfalfa in the orchards in order to keep a more uniform humidity.

Lloyd ('20a) found that a water deficiency was the cause of a large amount of abscission of the young fruits of *Juglans californica quercina*. He found also while working with cotton that a shower of rain on newly opened flowers caused some injury which ultimately led to abscission, and attributed the injury to a lack of pollination due to the rain. He suggested that the shedding of young bolls might be due to a competition for moisture, the older bolls robbing the younger ones in the case of a deficiency. Mason ('22) suggested that there should be a regulation of growth at or during the time of anthesis. He also observed that a low light

intensity, accompanied by low evaporation rates and day rains, is often the cause of abscission of forms. Martin and Loomis ('23) tried an irrigation experiment but failed to solve the problem, showing that there are factors other than the moisture content of the soil causing the abscission of forms in cotton. Detjen ('26) made a thorough examination of the bolls shed and found that bolls with no fertile seeds were shed immediately after flowering and were of little significance. Embryo abortion seemed to be the chief cause of the dropping of immature fruits. Namikawa ('26), working with a great variety of plants, and Dutt ('28), on the morphology of abscission in cotton, have contributed to the knowledge of the abscission of floral organs.

Methods

EXPERIMENT I

In the present investigations cotton seeds were treated either with 75 per cent H_2SO_4 or 1 per cent $HgCl_2$ for 30 minutes, after which they were thoroughly washed and soaked in tap water for 2 hours. They were then planted in a 1:6 sand and loam mixture, to which a little ground sphagnum had been added. The soil was sterilized by autoclaving for 2 hours. Most of the seeds were planted in 4–5-inch pots, but some in flats from which they were transplanted to pots while very small seedlings. While the major planting was on December 17, a few were planted three days later.

After germination the seedlings were divided into three equal groups, as follows: group 1 was grown under normal daylight, group 2 under a sixteen-hour day, and group 3 under continuous light. The normal daylight was supplemented by electric light produced by 300-watt incandescent lamps placed about 12 inches from the tops of the plants. All plants were kept as nearly as possible at the same temperature, 25 to 30° C., and all were given the same amount of water. These conditions were continued until February 3, when two distinct series were developed from the three existing sets. Series I was kept at a usual temperature of 20–24° C., while series II was kept as near as possible at 30–34° C. in another greenhouse compartment. Conditions permitting a more constant temperature would have been more desirable. Each series was subdivided twice as shown in table 1, first into

divisions 1, 2 and 3, on the basis of the length of day, and second into subdivisions A and B, for different conditions of soil moisture. Subdivision A received less water than B.

TABLE I

THE ARRANGEMENT OF THE EXPERIMENTAL PLOTS BEFORE MARCH 13 AS TO THE TEMPERATURE, LIGHT, AND MOISTURE, AND THE NUMBER OF PLANTS IN EACH PLOT

	1		2		3	
	12-hour day		16-hour day		24-hour day	
	A Low Moist.	B High Moist.	A Low Moist.	B High Moist.	A Low Moist.	B High Moist.
I 20–24° C.	20 plants	20 plants	20 plants	20 plants	20 plants	20 plants
II 30–34° C.	20 plants	20 plants	20 plants	20 plants	20 plants	20 plants

The plants were grown under these conditions until March 13, when subdivision B was modified as shown in table II. The extra plants used in the new grouping were miscellaneous plants grown for the most part in ordinary daylight and a temperature of 30–34° C. Subdivision A was continued without modification until the end of the experiment except that all plants were given an equal amount of moisture after March 13, since sufficient room was not available to carry the third subdivision which would have been desirable. This change from one length of day to another was intended to show the effects of a sudden change of length of day on the growth rate and on the development of flowers and the setting of bolls. At this time (March 13) all divisions except II–3 had developed squares, some more than others.

Division 4, which was made up from I–1–B, I–2–B, I–3–B and 10 plants from a general stock, was given 8 hours light per day (table II). Division 5, which was made up from the same sources as 4 (shown in table II), was given a 4-hour day. During the dark period both 4 and 5 were kept in a common dark room which had a temperature of about 25° C. One-half of each division was

kept in the warm room and one-half in the cold room during the light period.

TABLE II

THE POSITION OF THE EXPERIMENTAL PLANTS AFTER THE CHANGES OF MARCH 13

	1		2		3		4	5
	12-hour day		16-hour day		24-hour day		8-hour day	4-hour day
	A*	B*	A	B	A	B		
I 20–24° C.	Cont.		Cont.		Cont.		(1–10) I–3–B (11–20) I–2–B	(1–10) I–2–B (11–20) I–3–B
II 30–34° C.	Cont.	(1–10) II–3–B (11–20) II–2–B	Cont.	(1–10) II–1–B (11–20) II–3–B	Cont.	(1–10) II–1–B (11–20) II–2–B	(1–10) I–1–B (11–20) Misc.	(1–10) Misc. (11–20) I–1–B

In each set indicated above, the figures in parentheses represent a group of ten plants which was changed from its original position in the series; division and subdivision of table I, indicated by the symbols below these figures.

* Previous to March 13 represented differences in moisture.

The height of each plant was taken on February 3 and 22, March 13, April 5 and 21. The squares, flowers, and bolls were counted daily, and a record was made of each form shed, the approximate age of the shed squares, and the age of the shed bolls from the time the flowers opened. The general growth habits were noted as well as other interesting features.

EXPERIMENT II

Cotton seeds of the variety Upland Big Ball were soaked in 75 per cent H_2SO_4 for ten minutes, washed thoroughly, and planted October 8 of the second year in nine-inch pots, in a rich sandy loam with a small amount of clay. The seedlings were grown at ordinary greenhouse temperature until October 22, at which time they were moved to separate compartments for the duration of the experiment.

The experimental greenhouse was divided into a number of adjacent compartments 6 x 18 feet in size, which opened out into a hallway along the west side. They were equipped with benches along both sides and across one end. These benches were 2½ feet wide along the sides and 3 feet along the end, giving ample room for the plants during the early stages of growth but crowding them somewhat towards the last. Two adjacent compartments were used, the outer one at the end of the greenhouse being for the lower-temperature plants. These compartments were steam-heated with a double coil under each bench along the side. In the higher-temperature room an extra double coil was installed to prevent a temperature drop during unusually cold nights.

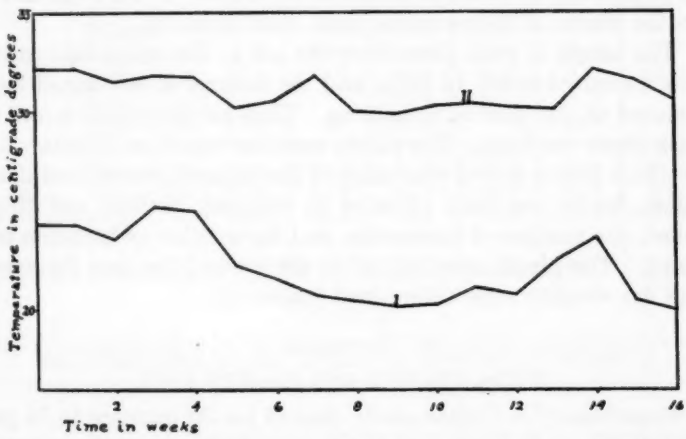

Fig. 1. Average weekly temperature of the two compartments as recorded four times daily. Compartment I, low temperature; compartment II, high temperature.

Compartment I was kept at an average temperature of 21.9° C. and compartment II at an average temperature of 30.9° C. Temperature readings were taken four times daily: 8 A.M., 1 P.M., 6 P.M., and 11 P.M. These readings were averaged into weekly temperatures as shown in fig. 1. The relative humidity was not measured but it appeared to be comparatively high most of the time, especially in the lower-temperature room.

One hundred plants were grown in each compartment. They

were divided equally into two sets A and B of 50 plants each. Set A received no light other than that of the short day of the winter months. Set B received approximately 20 hours of light each day, the daylight being supplemented by incandescent lamps of 300 watts. These lamps were arranged above the plants so as to give a good distribution of light and not affect the plants too greatly by temperature differences. A and B were separated at night by heavy canvas curtains. The curtains did not keep out all of the light but the small amount of reflected light was of low intensity.

The plants were arranged in three rows on the benches. At the time of measuring, every 14 days, they were shifted systematically as to position in order to minimize differences in the local effects on the plants of temperature, light, and humidity.

The height of each plant from the soil to the apical bud region was measured every 14 days, and the squares as developed were counted at the time of measuring. Thus an individual record of each plant was kept. The plants were harvested on February 14, at which time a record was taken of the squares present and those fallen, leaves one inch or more in diameter present and those fallen, the number of internodes, and the number of branches per plant. The plants were cut off at the ground line and the green and dry weights were determined (table VI).

RESULTS

TOTAL GROWTH AND GROWTH RATE

Experiment I.—Cotton seeds treated for 30 minutes in 75 per cent H_2SO_4 or in 1 per cent $HgCl_2$ gave higher percentage germination than untreated seeds. The young seedlings from seeds treated in H_2SO_4 or $HgCl_2$ showed little or no signs of damping off or wilt diseases when planted in sterilized soil. They grew more rapidly and withstood the low temperature with less injury than the untreated seedlings. Some seedlings, however, showed signs of injury.

The different lengths of day and the differences in temperature were correlated with marked differences in the growth of the cotton plants. The amount of growth was proportional to the length of day when the temperature and moisture were the same

(table III and pl. 46, figs. 1 and 2). The growth rate increased with the increase in length of day.

TABLE III

THE AVERAGE HEIGHT IN INCHES OF EACH SET OF 20 PLANTS TAKEN ON THE RESPECTIVE DATES

	Date	1		2		3	
		12-hour day		16-hour day		24-hour day	
		A Low Moist. Ht.	B High Moist. Ht.	A Low Moist. Ht.	B High Moist. Ht.	A Low Moist. Ht.	B High Moist. Ht.
I 20–24° C.	February 3	8.7	8.3	10.4	11.3	11.8	12.9
	February 22	9.5	10.0	11.0	12.4	12.8	13.8
	March 13	10.7	11.0	12.5	14.0	15.0	15.0
	April 5	11.7		14.4		17.0	
	April 21	11.9		15.0		18.8	
II 30–34° C.	February 3	9.3	8.8	11.3	11.9	12.1	13.0
	February 22	11.3	11.9	13.1	16.7	15.5	18.8
	March 13	14.0	15.4	15.1	20.5	21.1	25.0
	April 5	17.5		26.5		30.8	
	April 21	19.4		30.8		34.0	

TABLE IV

THE AVERAGE HEIGHT IN INCHES OF EACH SET OF 20 PLANTS TAKEN ON THE RESPECTIVE DATES, AFTER THE CHANGES OF MARCH 13

	Date	1		2		3		4	5
		12-hr. day		16-hr. day		24-hr. day		8-hr. day	4-hr. day
		A* Ht.	B* Ht.	A Ht.	B Ht.	A Ht.	B Ht.	Ht.	Ht.
I 20–24° C.	March 13	10.7		12.5		15.0		15.1	14.0
	April 5	11.7		14.4		17.7		16.2	15.5
	April 21	11.9		15.0		18.8		16.8	17.1
II 30–34° C.	March 13	14.0	22.7	16.9	19.7	21.1	18.5	10.7	10.2
	April 5	17.5	27.2	26.5	24.8	30.8	25.9	12.3	11.6
	April 21	19.4	29.7	30.8	26.7	34.0	28.3	13.8	13.8

* Previous to March 13 represented differences in moisture.

The appearance of the plants was very similar except that the plants of the longer day spread less in proportion to the height (pl. 46, fig. 2). The 24-hour-day plants at the higher temperature produced very few fruiting spikes, most of which were more like the ordinary vegetative branches. The plants at the lower temperature assumed a drooping appearance, whereas those at the higher temperature spread their leaves in the usual form. The plants at the lower temperature were more stocky. The total growth was practically twice as great at the higher temperature as at the lower temperature with the same length of day

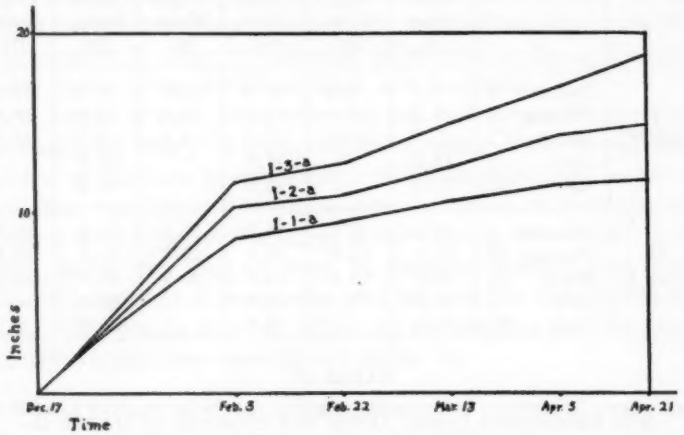

Fig. 2. The total growth in inches of the low-temperature plants plotted against the time. I-1-a, 12-hour-day plants; I-2-a, 16-hour-day plants; I-3-a, 24-hour-day plants.

(table III and pl. 46, fig. 3, and pl. 47). The growth rate was more regular at the lower temperature than at the higher temperature, giving a smoother growth curve. The difference in growth between the plants with higher and lower moistures was greater at the higher temperature (table III). Each division at the higher temperature showed considerably more growth with high moisture. The 12- and 16-hour-day plants at low temperature showed more growth with high moisture, whereas the 24-hour-day plants showed similar growth at high and low moistures (table III). The plants given less moisture shed more leaves than

those given the larger amount, especially those at the higher temperature.

The plants changed from a 12- to a 16-hour day showed a slight increase in growth rate for a few days, followed by a decline (fig. 4, section 1). Plants changed from a 12- to a 24-hour day

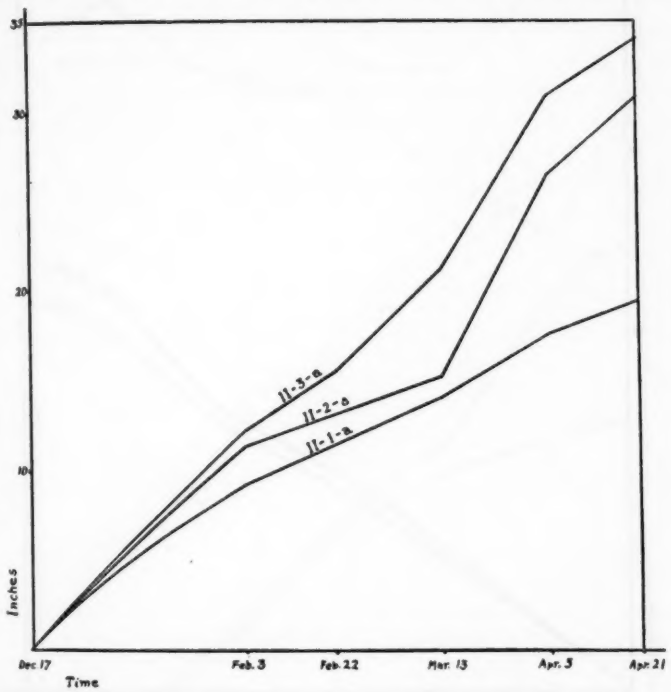

Fig. 3. The same as fig. 2 except the plants were grown at the higher temperature. II-1-a, 12-hour-day plants; II-2-a, 16-hour-day plants; II-3-a, 24-hour-day plants.

showed a marked increase in growth rate which was followed by an abrupt decline (fig. 4, section 1). Plants changed from a 16- to a 12-hour day showed a steady decline in growth for some time, followed by a very sudden increase accompanied by fruiting (fig. 4, section 2). Plants changed from a 16- to a 24-hour day showed an increase of growth rate followed by the usual decline (fig. 4, section 2). Plants changed from a 24- to 12- and 16-hour

days showed a steady decline in growth rate (fig. 4, section 3). The decline in growth rate after April 5 correlated with the usual decline of the cotton plant at this stage of development.

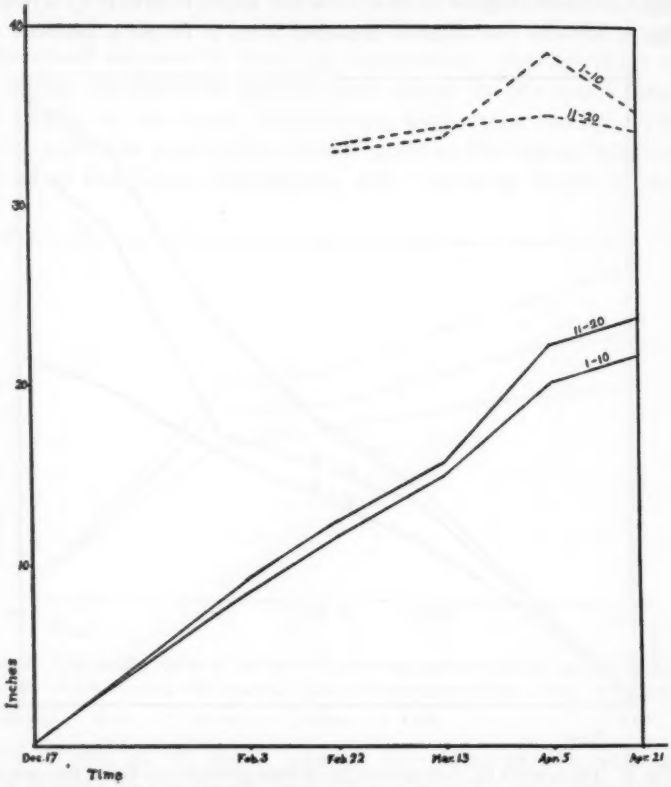

Fig. 4, section 1. The continuous lines represent the total growth in inches of the high-temperature plants changed from a 12- to 16- and 24-hour days plotted against the time. Plants 1–10 were changed from a 12- to a 16-hour day, whereas 11–20 were changed from a 12- to a 24-hour day.

The broken lines represent the growth per day of these plants plotted against the time. (Interchange numbers 1–10 and 11–20 in broken lines.) The growth rate is in hundredths.

Plants changed from 24- and 16-hour days to an 8-hour day showed a decline in growth rate. Those changed from a 12-hour

day to an 8-hour day showed an increase in growth rate. Groups of plants changed from the 12-, 16- and 24-hour days to a 4-hour day showed an increase in growth rate accompanied by chlorosis.

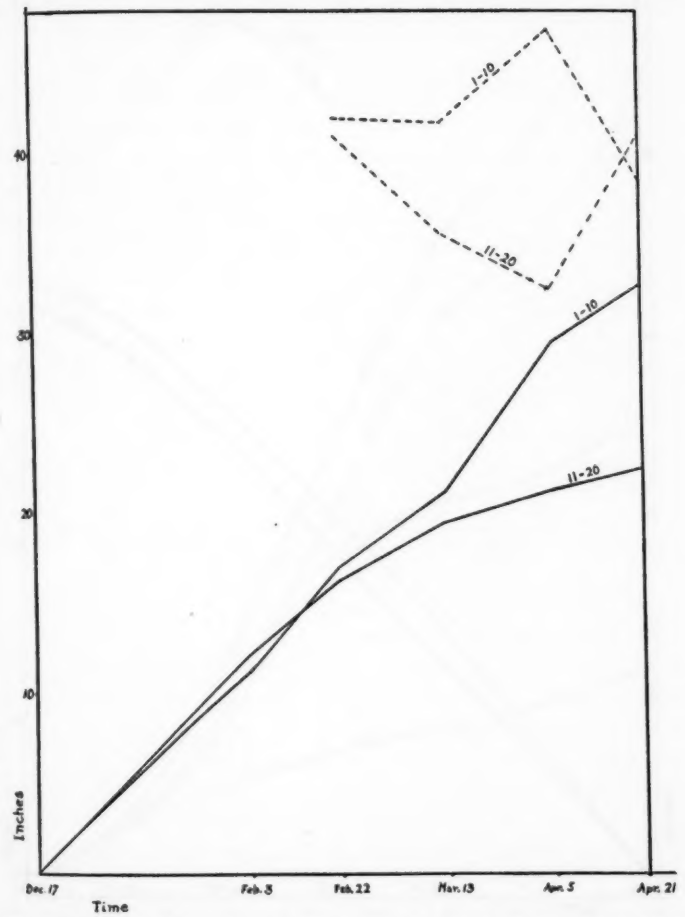

Fig. 4, section 2. The same as section 1, except plants 1–10 were changed from a 16- to a 24-hour day, and 11–20 from a 16- to a 12-hour day.

Experiment II.—As in experiment I, the amount of growth in height was proportional to the length of day at a given tempera-

ture (table v). At the same length of day the growth increased enormously with 9° C. difference in average temperature; the

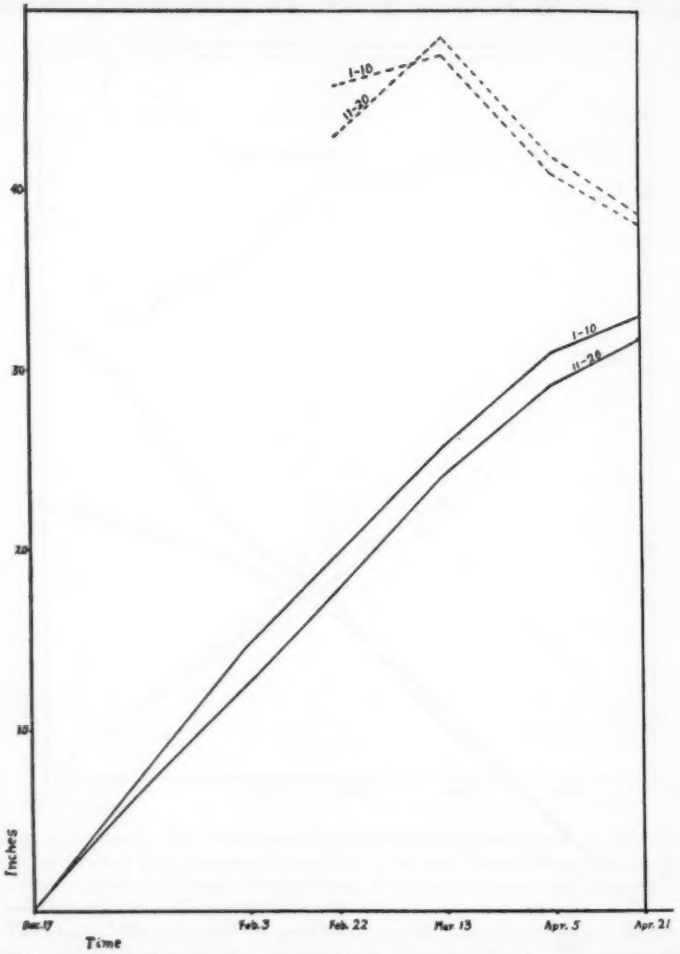

Fig. 4, section 3. The same as sections 1 and 2 except plants 1–10 were changed from a 24- to a 12-hour day, and 11–20 from a 24- to a 16-hour day.

plants grown at a higher temperature being more than twice as tall as those at a lower temperature and approximately twice as

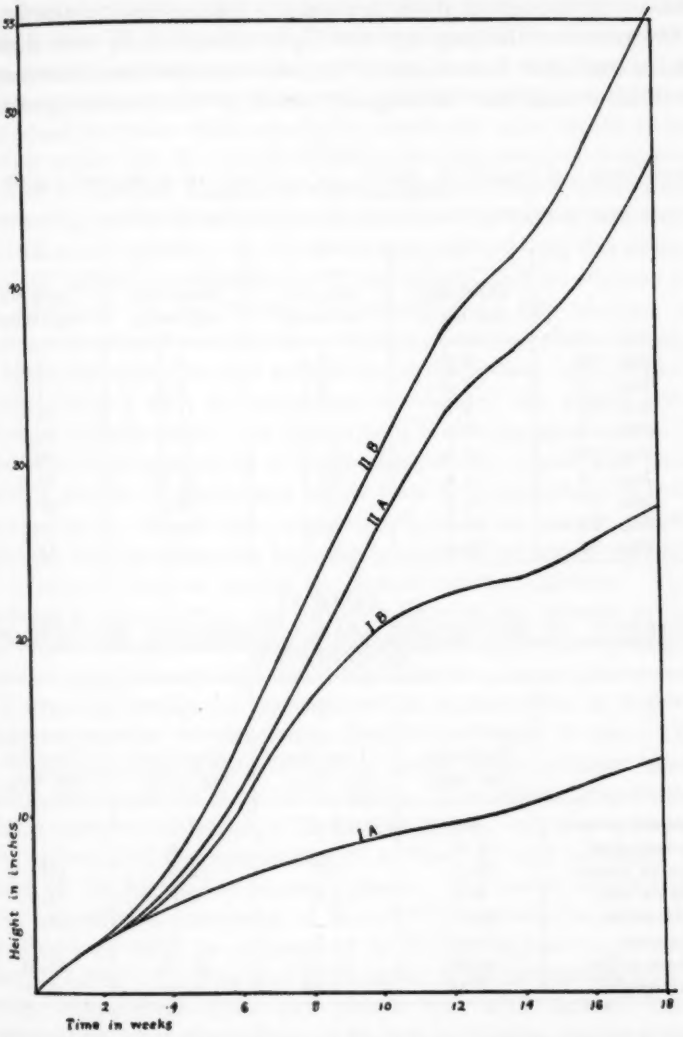

Fig. 5. Curves showing the growth in height of the respective sets; IA, lower temperature-short day plants, IB, lower temperature-long day plants, IIA, higher temperature-short day plants, and IIB, higher temperature-long day plants, as plotted in inches against the time in weeks.

heavy, both in green and in dry weight (tables v and vi, and fig. 5). The plants of the long day and higher temperature were slightly more succulent than those of the lower temperature; however, it will be noticed that the long-day plants of the lower temperature

TABLE V

THE AVERAGE HEIGHT IN INCHES OF EACH SET OF 50 PLANTS TAKEN ON THE RESPECTIVE DATES

	IA	IB	IIA	IIB
	Short day, low temp.	Long day, low temp.	Short day, high temp.	Long day, high temp.
Oct. 25	3.2	3.1	3.4	3.3
Nov. 8	4.2	5.7	6.2	7.4
Nov. 22	6.6	10.8	11.6	14.1
Dec. 6	7.9	16.5	18.9	22.4
Dec. 20	8.9	20.6	26.2	30.1
Jan. 3	9.6	22.5	32.8	37.5
Jan. 17	10.4	23.4	36.2	41.6
Jan. 31	11.9	25.3	40.5	48.0
Feb. 14	13.1	27.5	47.3	55.7

TABLE VI

STATISTICS OF THE SQUARES, LEAVES, INTERNODES, BRANCHES, AND GREEN AND DRY WEIGHTS PER PLANT TAKEN AT THE END OF EXPERIMENT II

	IA	IB	IIA	IIB
	Short day, low temp.	Long day, low temp.	Short day, high temp.	Long day, high temp.
Squares present	5.8	1.5	3.1	
Squares shed	1.2		.2	
Leaves present	16.9	9.5	18.3	19.4
Leaves shed	6.2	12.6	4.7	4.8
Internodes	18.	21.	23.	23.
Branches	6.1	1.9	.9	.3
Green weight	16.90	24.09	37.75	42.54
Dry weight	3.14	4.34	6.92	7.75

lost a large percentage of their leaves which affected both their green and dry weights (table vi). As shown by the growth curves (fig. 5) the growth rate was relatively low and approximately the same in all sets for the first two weeks. This is no doubt due to

the similar conditions of the early growth of the plants. The growth rate of the plants of the short day and lower temperature did not increase materially whereas that of the plants of a longer day and especially those of the higher temperature showed a gradual increase which reached a maximum after about three to four weeks (fig. 5). About 95 days after planting, all four sets showed a decrease in growth rate. At this time squares were developing on the lower temperature-short day plants and were noticed some days later on the lower temperature-long day plants and the higher temperature-short day plants, but no squares at all developed on the higher temperature-long day plants. A decrease in growth rate has been found to occur at about the age of flowering under normal conditions of the cotton belt, in both Experiments I and II, regardless of whether the plants were developing fruits or not. In Experiment II this decrease in growth rate was accompanied by a longer natural day which also gave light of greater intensity and an increase in temperature in both rooms (fig. 1). It is evident, therefore, that the decrease in growth rate was not due to poorer growing conditions but more probably to a natural decline during the period of reproduction. This decrease in growth rate was followed later by an increase as the days became longer and the light of greater intensity, even though the temperature was kept somewhat lower.

Except for height the general contour of the plants at a given temperature was the same regardless of the length of day. The lower-temperature plants developed larger leaves, shorter internodes, and in general were more stocky. The higher-temperature plants were more slender, with smaller leaves, longer internodes, and approached the appearance of a vine. It was necessary to stake up the higher-temperature plants. The lower-temperature plants developed an average of 18 and 21 internodes respectively, as compared with an average of 23 in the higher-temperature plants (table VI). The average length of the internodes on the lower temperature-short day plants was 0.72 inches, lower temperature-long day plants 1.28 inches, higher temperature-short day plants 2.05 inches, and higher temperature-long day plants 2.42 inches.

No fruiting branches developed on the higher temperature-long

day plants and few vegetative branches (table VI). The branches developed were for the most part on plants whose growing point had been injured, in which case one branch became dominant, taking the place of the main stem. Few fruiting branches of any size had developed on the higher temperature-short day plants and the lower temperature-long day plants, but a great number were observed in the early stages of development. The lower temperature-short day plants developed a number of fruiting branches and a few vegetative branches.

THE DEVELOPMENT OF FRUITS

Experiment I.—The appearance of squares varied considerably with the difference in length of day as well as with a difference in temperature. The first squares were noticed February 18 on the 12-hour-day plants in both the low- and the high-temperature sets. The plants receiving more moisture were the first to show a tendency to fruit but the difference was of little significance. The 16-hour-day plants produced at about the same time a few minute squares which were shed immediately, and no permanent squares appeared on the low-temperature plants until the first of March. The 16-hour-day plants at the high temperature shed the squares as rapidly as they appeared until near the middle of April; therefore, there were very few of any size when the experiment was concluded. The 24-hour-day plants at the low temperature developed permanent squares the first of March. The high-temperature plants with the exception of one individual did not develop permanent squares. The one exceptional plant produced one square which flowered on April 22, but it did not appear until after the temperature was allowed to go down below 25° C., due to a lack of heat in the greenhouse.

The first flowers appeared on the 12-hour-day plants at the high temperature on March 13. A number of flowers appeared in rapid succession until one or more bolls were set on each plant, after which a great number of the existing squares was shed either before or after flowering and new ones ceased to develop. The low-temperature plants did not produce flowers until April 1 after which time many flowers appeared in rapid succession and few squares and no bolls were shed before April 21. The first

flowers appeared on the 16-hour-day plants in the low-temperature group on April 4. Fewer flowers appeared on the 16-hour-day plants but bolls were set and grew very rapidly. No flowers appeared on the 16-hour-day plants at the high temperature. The first flower opened on the plants exposed to a 24-hour-day and low temperature April 21, but a number were opening and fruits were set within the next three days (pl. 46, fig. 1a).

Plants changed from a 12- to a 16-hour day continued to produce squares and to set fruits. The plants changed from a 12- to a 24-hour day did not produce so many flowers and in some cases abscissed all squares present, failing to set fruits at all. The fruits set were for the most part smaller and on longer branches than those of the 12- or the 16-hour-day plants.

TABLE VII

THE NUMBER OF SQUARES PER PLANT AND THE PER CENT SHED FROM THE TIME THE FIRST SQUARES APPEARED UNTIL MARCH 13

		1		2		3	
		12-hr. day		16-hr. day		24-hr. day	
		A Low Moist.	B High Moist.	A Low Moist.	B High Moist.	A Low Moist.	B High Moist.
I 20–24° C.	Sq. per plant	1.65	1.15	1.05	.95	.25	
	Sq. shed per plant	.1	.75	.25	.95	.1	.15
	Total forms per plant	1.75	1.9	1.3	1.9	.35	.15
	Per cent sq. shed	5.77	39.4	19.2	52.77	28.57	100.0
II 30–34° C.	Sq. per plant	2.45	2.55	.15	.15		
	Sq. shed per plant	.9	.95	.2	.35		
	Total forms per plant	3.35	3.5	.35	.5		
	Per cent sq. shed	26.85	27.14	57.14	70.0		

Plants changed from a 16- to a 12-hour day did not produce squares until March 26, but they flowered immediately thereafter, and soon all plants bore rapidly growing squares and a number bore fruits. Plants changed from a 16- to a 24-hour day, contrary to the general rule, produced squares as early as March 21. In a large number of plants all the squares were shed but in a few cases flowers opened and fruits were set. The plants setting

fruits were among those showing the highest growth rate at the time.

TABLE VIII

STATISTICS OF THE SQUARES, FLOWERS, AND BOLLS PER PLANT, AND OF ALL FORMS SHED THROUGHOUT THE EXPERIMENT. THE POSITIONS OF THE PLANTS ARE SHOWN AS THEY EXISTED FROM MARCH 13 TO APRIL 21

		1		2		3		4	5
		12-hr. day		16-hr. day		24-hr. day		8-hr. day	4-hr. day
		A*	B*	A	B	A	B		
I 20–24° C.	Sq. per plant	2.2		1.75		2.35		2.1	
	Fl. per plant	.25		.15					
	Bolls per plant	1.35		.8					
	Sq. shed per plant	2.85		2.85		1.55		1.7	1.5
	Total forms per plant	6.65		5.55		3.65		3.8	1.5
	Per cent sq. shed	42.85		51.35		39.74		44.15	100.0
II 30–34° C.	Sq. per plant	1.7	2.45	2.5	.65	.15	.4	2.3	
	Fl. per plant	.2							
	Bolls per plant	1.7	.15		.55		.51		
	Sq. shed per plant	5.65	.15	1.6	3.4	.45	4.55	1.6	1.4
	Fl. shed per plant	.1							
	Bolls shed per plant	.75			.65		.55		
	Total forms per plant	10.1	2.75	4.1	5.25	.6	6.01	3.9	1.4
	Per cent sq. shed	55.94	5.45	39.02	64.76	75.0	75.7	41.02	100.0
	Per cent flowers shed	3.77							
	Per cent bolls shed	27.65			54.16		51.88		

* Previous to March 13 represented differences in moisture.

The plants changed from a 24- to a 12-hour day developed squares, beginning April 8. No flowers opened before the experiment closed. Those changed from a 24- to a 16-hour day did not develop normal squares before the experiment was closed.

One or two vestigial squares appearing on one plant were immediately shed.

All squares present on the plants changed from the 12-, 16-, and 24-hour day to a 4- and 8-hour day were immediately shed. The 4-hour-day plants did not produce more squares. The 8-hour-day plants produced a second set of squares which grew very slowly and often showed a tendency to spread their bracts long before they were large enough to open. No flowers appeared. The results were due no doubt to the combination of a change in temperature and a change in the length of day.

Table VII shows the number of squares on each plant, the number shed, and the percentage of all squares shed up until March 13. Table VIII shows the number of squares, flowers and bolls per plant, and the percentage of each shed by the end of the experiment. The position of the plants is shown after the changes of March 13, but the individual calculations were made from the beginning until the experiment was closed April 21.

Experiment II.—The first squares were noticed on the lower temperature-short day plants on January 2. They developed slowly and did not open until near the middle of February. Squares were observed on the lower temperature-long day plants and higher temperature-short day plants the first week in February. No flowers were produced but some squares on the short day-higher temperature plants grew very rapidly and were showing signs of opening by February 14. No squares were developed on the higher temperature-long day plants.

When the plants were harvested on February 14 a single plant from the long day-higher temperature plants, which was of unusual height, was set to one side and kept growing under ordinary greenhouse conditions. The first squares were observed on May 15, and measurements at this time showed that the plant had grown only two inches in the last three months as compared with 76 inches in the four months under the higher temperature-longer day conditions.

Discussion

Much emphasis has been placed on relative day length as one of the most important factors in the natural environment of the plant. Not until recently has much attention been given to the

periodic responses of plants grown under various day lengths, as affected by variations in temperature. Due to the great complexity of environmental combinations, it is reasonable to assume that no one factor is responsible for a given response under all conditions. Temperature is recognized as a limiting factor in the zonation of plants, as well as in the process of photosynthesis (Blackman, '05). It has been shown by Gilbert ('26b) that temperature variations are important in the time of development of flower primordia and may be as effective as different day lengths, on some plants. Cotton is very sensitive to temperature changes as shown by Fung ('11), and therefore it is reasonable to expect that temperature would greatly modify the effects of the different lengths of day on the development of fruits and the percentage of fruits matured, as well as the vegetative growth. Similarly, under different combinations of factors, moisture or intensity of light might play the same role.

Gilbert suggests that under suitable environmental combinations specific temperatures may be found to substitute for relative day lengths in the development of flower primordia. This seems to be the case with the cotton plant. What determines the initiation of flower primordia is not known. Loew ('27) states that these responses are dependent on the presence of certain quantities of phosphoric acid as well as certain concentrations of the simple sugars in the cell sap. Gilbert ('26b) found under both low- and high-temperature conditions that the ratios of total carbohydrates to total nitrogen, and soluble carbohydrates to soluble nitrogen, were distinctly ascending as flower primordia are formed. What relation temperature changes and day length have on the formation of the necessary concentrations of these constituents is not known, but it is evident that flowering will take place under different combinations of conditions.

Sudden changes in the day length during the flowering period of the plant showed somewhat different results, and it was impossible with the small amount of data to determine when the flowering was initiated. It is possible that the flowering primordia are initiated but suppressed in their development under such conditions as higher temperature and long day, and when changed to a shorter day are allowed to develop, though somewhat slowly.

On the other hand, when the length of day is increased, the development is more rapid. Plants grown under a 4-hour day do not appear to be able to initiate flowering. It would seem that a very low percentage of carbohydrates would develop in plants under these conditions.

Branetzky ('97) observed that plants grow more slowly by day than by night. Balls, Lloyd, and others found that the cotton plant not only grows more slowly by day but that it shrinks a little during the hot part of the day. The shrinking is no doubt due to the loss of water by the rapidly transpiring cells under the temperature and humidity conditions of the noon-day sun. The cells in the region of the growing point are very elastic and will vary in size with the water content. This may limit the size of the cells maturing at this time, as the mature cell is more rigid and tends to plasmolyze instead of shrink with the loss of water. It is evident, however, that light has an effect on the development of the plant other than that of photosynthesis as shown by Went ('25, '28). The elongation and expansion of the cell wall are somewhat dependent on its stretching, due to turgidity. The nature of the stimulus of light on limiting this expansion is not known, but it appears to be other than the reduction of the water content. This stimulus appears to vary with the intensity of the light but no accurate measurements have been made under carefully controlled conditions. The limiting effects may be a direct relation of the absorption of the light rays by the protoplasm or an interrelation of the moisture and temperature of the cell as affected by the absorption of the light energy. Shortening the length of day as well as decreasing the intensity of the sunlight will affect the size and contour of the cells and the development of the leaves, stems and roots, as shown by Johannson ('27) and Doroshenko ('27). Since thicker leaves are produced by bright sunlight one would be led to believe that the sun has a direct influence other than temperature changes.

It is very difficult to determine at what length of day the light is more efficient per hour of illumination. If we judge by the elongation of the plant we must remember that plants tend to elongate more in proportion under lights of lower intensities, becoming extremely spindling if kept in the dark. If we choose

the development of fruits as a criterion we find that this does not necessarily correlate with the greatest vegetative growth. Lubimenko and Szeglova ('28) found that light was more efficient during an 8-hour day on cotton plants as measured by vegetative growth. In these experiments the greatest efficiency as measured by elongation was found to be at the long day, but as measured by dry weight, at the short day. This is evidenced by the more stocky appearance of the short-day plants (pl. 46, fig. 3) and the greater number of leaves per unit length (table VI). It must be borne in mind that the short-day plants are exposed to the light during the part of the day when the natural light is at its maximum intensity. It is probable that the more intense light of the noon-day is above the optimum for photosynthesis, but it has been shown by Johannson ('27) that light of this intensity stimulates the development of thicker leaves and a more stocky plant. On the other hand, the electric light is no doubt below the optimum for greatest efficiency in the production of photosynthates. It is evident, however, that these responses are greatly modified by differences in temperature and moisture (pl. 46, fig. 1 as compared with fig. 2).

SUMMARY

1. Two experiments in successive years were made on the effects of the variations in temperature, moisture, and length of day, on the growth and reproduction of cotton.

2. In experiment I, cotton plants were grown in 4–5-inch pots under a 12-, 16- and 24-hour day, with variations in moisture and temperature. In experiment II, cotton plants were grown in 9-inch pots under a 12- and 20-hour day with a variation in temperature.

3. In experiment I a number of plants was changed from one length of day to another during the fruiting period.

4. The growth in height increased with the increase in length of day and was more than twice as great at the higher temperature.

5. All the plants grown at the lower temperatures produced squares, and in most cases set fruits regardless of the length of day. The short-day plants at higher temperature produced squares and set fruits. The long day-higher temperature plants, with the exception of one individual, did not produce squares.

6. Plants changed to a shorter day showed a decrease in growth rate followed in some cases by an increase after a few weeks. Plants changed to a longer day showed an increase in growth rate followed by a decline.

7. In most instances the growth rate declined during the fruiting season. Plants that did not produce fruits showed a similar decline in growth rate.

8. The plants changed to a longer day produced flowers and fruits in a short time. Plants changed from a 16- and 24-hour day to a 12-hour day produced squares and some set fruits after a few weeks. Plants changed from 12-, 16- and 24-hour days to an 8-hour day shed all squares present but developed new ones after some weeks. Plants changed from a 24- to a 16-hour day and from 12-, 16- and 24-hour days to a 4-hour day did not produce normal squares before the experiment closed.

9. Although the cotton plant may produce fruits more readily with a specific day length, thus being classed as a medium-day plant, one is led to believe from the above data that temperature differences may be substituted for day lengths in certain combinations. Fruiting may occur in the cotton plant under any given day length of 8 hours or above, providing the temperature and other factors are favorably adjusted.

The author wishes to express his gratitude to Dr. E. S. Reynolds of the Henry Shaw School of Botany, Washington University, for the numerous suggestions and criticisms during the progress of this work, and to Dr. G. T. Moore, Director of the Botanical Garden, for the use of the library of the Missouri Botanical Garden.

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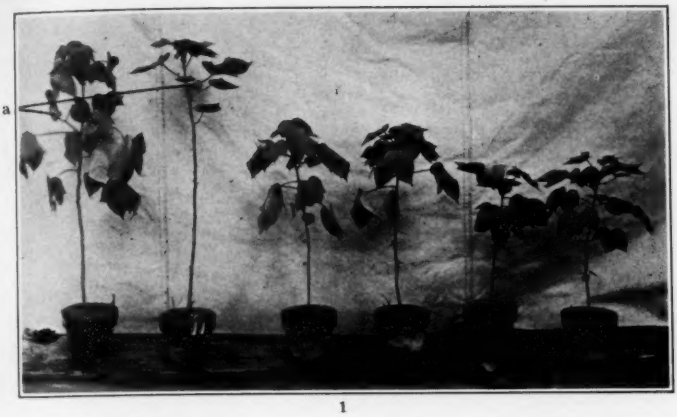
Explanation of Plate

PLATE 46

Fig. 1. Showing the effects of the difference in the length of day on the growth of cotton at low temperature; *a*, bolls. Two plants at the extreme right, 12-hour-day plants; two in the center, 16-hour-day plants; two at the extreme left, 24-hour-day plants.

Fig. 2. Showing the effects of the difference in the length of day on the growth of cotton at high temperature. Two plants at the extreme right, 12-hour-day plants; two in the center, 16-hour-day plants; two at the extreme left, 24-hour-day plants.

Fig. 3. Showing the effects of different temperatures on the growth of 12-hour-day plants.



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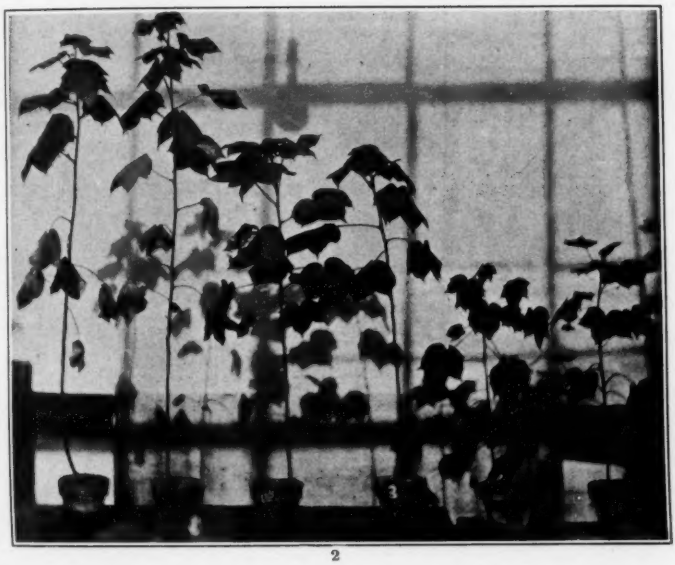

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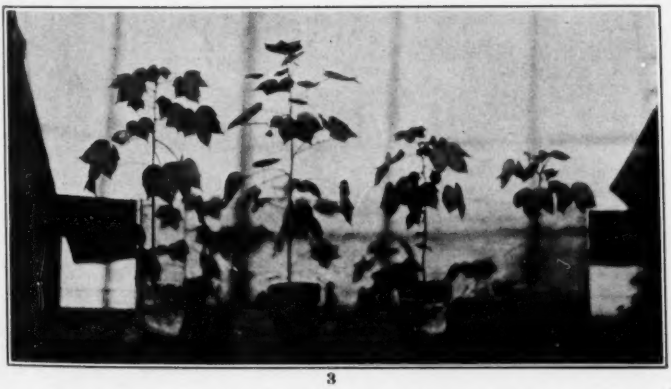


3

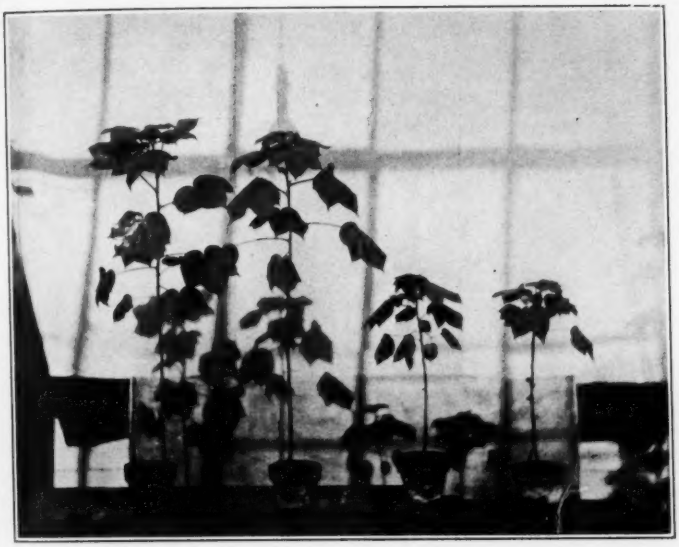
BERKLEY—EFFECTS OF DIFFERENT LENGTHS OF DAY AND TEMPERATURES

Explanation of Plate

PLATE 47

Fig. 1. Showing the effects of different temperatures on 16-hour-day plants. Two plants at the extreme right, low-temperature plants; two at the left, high-temperature plants.

Fig. 2. Showing the effects of different temperatures on 24-hour-day plants. Two plants at the right, low-temperature plants; two at the left, high-temperature plants.



1

2

BERKLEY—EFFECTS OF DIFFERENT LENGTHS OF DAY AND TEMPERATURES

GENERAL INDEX TO VOLUME XVIII

New scientific names of plants and the final members of new combinations are printed in **bold face** type; synonyms, and page numbers having reference to figures and plates, in *italics;* and previously published names and all other matter, in ordinary type

A

abietinus (Polystictus), 470
Abietis (Trametes), 474
Acidity of Missouri soils and its relation to plant distribution, A study of, 41
Adansonia, 128
adustus (Polyporus), 471
Agaricaceae, 470
Agaricus boletiformis, 391; *hirsutus*, 391; *saepiarius*, 391; *trabeus*, 392
Aleurodiscus disciformis, 485; **diffissus**, 485
Allium stellatum, Chromosome complements of, 465, *468;* Moly, 465; ursinum, 465
Alpova, a new genus of Rhizopogonaceae, with further notes on Leucogaster and Arcangeliella, 457
Alpova cinnamomeus, 461, *464*
Althaea, 118, 125
ambiens (Helicoma), 10
ammonis (*Gyroceras*), 1, 7
ammonis (Sarcopodium) (Tricholeconium), 7
amorphus (Polyporus), 471
anastomosans (Helicoma), 13, 14, *16*
Anderson, Edgar. The chromosome complements of Allium stellatum and Nothoscordum bivalve, 465; and Dorothea De Winton. The genetic analysis of an unusual relationship between self-sterility and self-fertility in Nicotiana, 97
Anechites adglutinata, 552
Anisolobus cognatus, 546; *Perrottetii*, 546
Antrodia mollis, 367
Apocynaceae of tropical America, New or otherwise noteworthy, 541
applicatus (Pleurotus), 470
Arcangeliella **australiensis**, 463; **Ravenelii**, 463; **rosea**, 462; **Stephensii**, 463
Arenaria cephaloidea, 284
arida (Coniophora), 485
Asclepiadaceae, New South American, 557
Asia, eastern, Hymenomycetous fungi of, 469
Aurantiporellus alboluteus, 333
auricula-Judae (Auricularia), 487
Auricularia auricula-Judae, 487; auriformis, 487
Auriculariaceae, 487
auriformis (Auricularia), 487
auriscalpium (Hydnum), 477
australiense (Hydnangium), 463
australiensis (Arcangeliella), 463
australiensis (*Octaviana*), 463

B

Bacteria, effect of ultra-violet radiation on, 18, 505
badio-ferruginea (Hymenochaete), 483
Beardsley, Martha L. The cytology of Funaria flavicans Michx. with special reference to fertilization, 509
bellus (Helicomyces), 10, 16
benzoinus (Polyporus), 471
Berkley, Earl E. Studies of the effects of different lengths of day, with variations in temperature, on vegetative growth and reproduction in cotton, 573
bicolor (Odontia), 479
biformis (Polystictus), 471
Bjerkandera adusta, 339, 340
Blepharodon **minimus**, 557; suberectus, 557
Boletus abietinus, 328; *adustus* 339, var. *crispus*, 340; *albidus*, 337; *applanatus*, 374; *arcularius*, 346, 394; *benzoinus*, 338; *caesius*, 337; *calceolus*, 358; *carpineus*, 339; *cinnabarinus*, 342; *cinnamomeus*, 354; *coccineus*, 342; *conchatus*, 384; *confluens*, 352, 353; *coriaceus*, 353; *crispus*, 340; *elegans*, 359; *ferruginosus*, 401; *fuliginosus*, 338; *fulvus*, 377, 383; *fuscoporus*, 339; *hirsutus*, 324; *ignitarius*, 382; *incarnatus*, 328; *incertus*, 397; *isabellinus*, 339; *lipsiensis*, 374; *marginatus*, 377; *medulla-panis*, 398; *nigromarginatus*, 324; *nummularis*, 359; *odoratus*, 370; *ovinus*, 350; *pelleporus*, 339; *perennis*, 353; *Pini*, 379; *pinicola*, 377; *pomaceus*, 383; *resinosus*, 338; *Rheades*, 336; *roseus*, 387; *salicinus*, 384; *semiovatus*, 377; *sistotremoides*, 347; *spumeus*, 345; *squamosus*, 356; *suberosus flabelliformis*, 339; *subtomentosus*, 353; *ungulatus*, 377; *varius*, 358; *versicolor*, 323
brisbanense (*Hydnangium*), 463
brumalis (Polyporus), 471
Bucholtz, Alexander F. The effect of monochromatic ultra-violet light of

measured intensities on behavior of plant cells, 489
Burt, Edward Angus. Hymenomycetous fungi of Siberia and eastern Asia—mostly of wood-destroying species, 469
byssoidea (Coniophora), 485

C

caesio-alba (Poria), 476
calcea (Sebacina), 487
Calcium content of higher plants, effect of ultra-violet radiation on, 565
Callirhoë, 125; *spicata*, 159, 165
Calyptospora columnaris, 293
Cantharellus alveolaris, 394
Capnorea pumila, 286
Card, H. H. A revision of the genus Frasera, 245
Carestianum (Septobasidium), 487
Celtidis f. *fulvescens* (*Gyroceras*), 4
Celtidis (*Gyroceras*), 2, 3
Celtidis (Helicoceras), 3, *8*
Celtidis (*Monilia*), 3
Chailletii (Stereum), 480
Chromosome complements of Allium stellatum and Nothoscordum bivalve, 465
cinnamomeus (Alpova), 461, *464*
citrinus (Leucogaster), 462
Cladomeris Schweinitzii, 347
Clarksville soils in Missouri, 48
Clavaria formosa, 486
Clavariaceae, 486
colliculosum (*Corticium*), 484
Colorado: The Polyporaceae of, 287; zones of vegetation in, 289
Coltricia cinnamomea, 354; *connata*, 353; *perennis*, 353; *tomentosa*, 349
commune (Schizophyllum), 470
confluens, (Corticium), 483
conformis (Ditiola), 486
confragosa (Daedalea), 475
Coniophora arida, 485; byssoidea, 485; olivacea, 485; **sibirica**, 485
Coremiella saxonicum, 7
Coriolellus Sepium, 366; *serialis*, 365
Coriolus abietinus, 328; *hexagoniformis*, 366; *hirsutulus*, 323; *Lloydii*, 326; *nigromarginatus*, 324; *pargamenus*, 330; *planellus*, 344; *prolificans*, 330; *subchartaceus*, 331; *versicolor*, 323; *zonatus*, 325
cornucopioides (Craterellus), 479
corticalis (Peniophora), 484
Corticium *colliculosum*, 484; confluens, 483; frustulosum, 483; galactinum, 483; hydnans, 484; illaqueatum, 484; investiens, 484; lactescens, 484; laeve, 484; ochraceum, 484; polygonium, 484; radiosum, 484; roseum, 484; sulphureum, 484
Cotton, Studies of the effects of different lengths of day, with variations in temperature, on vegetative growth and reproduction in, 573
Craterellus cornucopioides, 479
Cryptoporus volvatus, 321
Cucumbers, effect of ultra-violet radiation: on growth, 27, 30, 568; on calcium content, 570; on phosphorus content, 569
Curtisii (Helicoma), 10
Cyanide, effect of, on cultures of Fusarium lini, 75
Cyclomyces, 314
Cytidia salicina, 486
Cytokinesis in the Mosses, 533
Cytology of Funaria flavicans Michx. with special reference to fertilization, 509, *556*, *538*, *540*

D

Dacryomycetaceae, 486
Dacryomyces abietinus, 292
Daedalea, 314, 376; aurea, 476; confragosa, 475; *epigaea*, 347; *heteromorpha*, 366; *mollis*, 367; *Pini*, 379; quercina, 476; *saepiaria*, 391; *trabea*, 392; unicolor, 476, var. *violacea*, 328
Dasyscypha Agassizii, 292; arida, 292
Day, effects of different lengths of, with variations in temperature, on vegetative growth and reproduction in cotton, 573
delectans (Polyporus), 472
De Winton, Dorothea, Edgar Anderson and. The genetic analysis of an unusual relationship between self-sterility and self-fertility in Nicotiana, 97
dichrous (Polyporus), 472
diffissa (*Peniophora*), 485
diffissus (Aleurodiscus), 485
Dipladenia **Achrestogyne**, 543; congesta, 544; fragrans, 545; glabra, 545; Martiana, 545; **oblongifolia**, 544; surinamensis, 545; **upatae**, 545; urophylla, 545
disciformis (Aleurodiscus), 485
Distribution of some Rocky Mountain plants, 283
Ditiola conformis, 486
Dodecatheon tetrandrum, 284
Dodge, C. W. **Alpova**, a new genus of Rhizopogonaceae, with further notes on Leucogaster and Arcangeliella, 457
dryadeus (Polyporus), 472

E

Echites *agglutinata*, 552; *circinalis*, 552; *coalita*, 552; *cognata*, 546; *Dusenii*,

552; *portobellensis*, 553; *Sanctae-Martae*, 555; *spectabilis*, 552
Eichleriella spinulosa, 487
Elfvingia applanata, 374; *lipsiensis*, 374; *megaloma*, 374
ellipticum (Helicoon), 11
Elytropus spectabilis, 552
Erinaceus (Hydnum), 477
Exidia glandulosa, 292, 487
Exolobus **marmoreus**, 561; patens, 562

F

Farlowii (Podaxis), 459, *464*
fasciatum (Stereum), 480
Favolus, 394; alveolaris, 394, *454; canadensis*, 394; *europaeus*, 394; *ohiensis*, 394; *striatulus*, 394
Femsjonia luteo-alba, 487
Fertilization: in Funaria flavicans Michx., 509, *536*, *538*, *540*; in the Mosses, 509; in the Liverworts, 511
fibrillosus (Polyporus), 472
Flax, glucoside content of, 66
floccosus (Leucogaster), 459
Fomes, 376; *Abietis*, 379; *alboluteus*, 333; annosus, 389, *450; applanatus*, 374; conchatus, 384; connatus, 396; Demidoffii, 385, 388, *446; Earlei*, 385; *Ellisianus*, 388; fraxinophilus, 389, f. Ellisianus, 388, *462; fuliginosus*, 338; fulvus, 383, *446*, 474; igniarius, 382, *448*, 474; *Juniperinus*, 385; *leucophaeus*, 374; *marginatus*, 377; *megaloma*, 374; *nigricans*, 382; nigrolimitatus, 380, *454;* ohiensis, 376; Palliseri, 474; Pini, 379, *442;* pinicola, 377, *444*, *446*, 474; *pomaceus*, 383; *ponderosus*, 378; putearius, 381; roseus, 372, 387, *436*, 474; scutellatus, 376; *tenuis*, 363; *ungulatus*, 377
Fomitopsis *annosa*, 389; *pinicola*, 377; *rosea*, 387
formosa (Clavaria), 486
Fossombronia, fertilization in, 512, 530
Frasera, A revision of the genus, 245
Frasera, 256; *albicaulis*, 269, f. *alba*, 269; albomarginata, 274, *280*, var. **induta**, 275; *ampla*, 259; *angustifolia*, 259; *carolinensis*, 262; caroliniensis, 262, *280;* coerulea, 271; *Cusickii*, 270; fastigiata, 264, *280; induta*, 275; *macrophylla*, 259; montana, 266, *280;* neglecta, 265, *280;* nitida, 267, *280*, var. **albicaulis**, 269, *280*, var. **Cusickii**, 270, *280*, *282; nitida* var. *albida*, 267; *officinalis*, 262; paniculata, 275, *280;* Parryi, 273, *280;* puberulenta, 272, *280*, *282;* speciosa, 258, *280; speciosa* var. *angustifolia*, 259, var. *scabra*, 259, var. *stenosepala*, 259; *stenosepala*, 259; *thyrsiflora*, 264; tubulosa, 271, *280; utahensis*, 275; *venosa*, 259; *verticillata*, 262; *Walteri*, 262
Friesii (Porothelium), 476
frondosus (Polyporus), 472
frustulosum (Corticium), 483
Fuller, Harry J. Stimulatory effects of radiation from a quartz mercury vapor arc upon higher plants, 17; F. Lyle Wynd and. Some effects of ultraviolet radiation upon the calcium and phosphorus content of higher plants, 565
Fulvifomes Juniperinus, 385
fulvus (Fomes), 474
Funalia stuppea, 368
Funaria flavicans Michx., cytology of, 509; hygrometrica, 512
Fusarium lini, 66
fuscopes (Helicomyces), 15
Fuscoporia ferruginosa, 401
fusco-violaceus (Irpex), 478
fuscum (Stereum), 481

G

galactinum (Corticium), 483
Ganoderma, 373; applanatum, 374, *442; leucophaeum*, 374; *lipsiensis*, 374
Gasteromycetes, 457
gausapatum (Stereum), 481
Genetic analysis of an unusual relationship between self-sterility and self-fertility on Nicotiana, 97
gibbosa (Trametes), 475
gigantea (Peniophora), 485
gilvus (Polyporus), 472
glabrum (Hydnangium), 463
glandulosa (Exidia), 487
Gloeophyllum abietinellum, 391; *hirsutum*, 370, 391; *pallidofulvum*, 393; *trabeum*, 393
Glucoside content of flax, 66
Goodman, George J. Notes on the distribution of some Rocky Mountain plants, 283
griseum (Helicosporium), 9
Guepinia monticola, 292
Gyroceras, 1; *ammonis*, 1, 7; *Celtidis*, 2, 3, f. *fulvescens*, 4; *divergens*, 4; *Nymphaearum*, 5; *Plantaginis*, 5; *saxonicum*, 7
Gyrocerus, 2

H

Haemadictyon circinalis, 552
Hagerstown soils in Missouri, 44
Helicoceras, The genus, 1
Helicoceras, 1; **Celtidis**, 3, *8;* **Nymphaearum**, 5, *8;* **Oryzae**, 2, 6, *8;* **Plantaginis**, 5, *8*
Helicoma ambiens, 10; **anastomosans**, 13, 14, *16;* Curtisii, 10; Morgani, 13, 14; olivaceum, 11; polysporum, 11; repens,

11; **tenuifilum**, 14, *16;* violaceum, 14; **Westoni**, 12, *16*
Helicomyces bellus, 10, 16; **fuscopes**, 15; roseus, 10, 16
Helicoon ellipticum, 11
Helicosporeae, Brief notes on the, with descriptions of four new species, 9
Helicosporium griseum, 9; lumbricoides, 10; lumbricopsis, 10; *Nymphaearum*, 5; phragmites, 9
Hesperalcea, 137, 214; *malachroides*, 128, 215
Hesperochiron pumilus, 284
Heterobasidion annosum, 389
heteromorpha (Lenzites), 470
Hexagona, 394; *alveolaris*, 394; *micropora*, 394; *Mori*, 394; *striatula*, 394
hirsutum (Stereum), 481
hirsutus (Polystictus), 471
hispida (Trametes), 474
hispidus (Polyporus), 472
Hollii (Hydnum), 477
Host plants for species of Polyporaceae, 294
Hydnaceae, 477
Hydnangium australiense, 463; *brisbanense*, 463; *glabrum*, 463; *nudum*, 462; *Ravenelii*, 463; *Stephensii*, 463, v. *Ravenelii*, 463; *virescens*, 462
hydnans (Corticium), 484
Hydnum auriscalpium, 477; Erinaceus, 477; Hollii, 477; **Murashkinskyi**, 477; ochraceum, 478; **reflexum**, 478; velutinum, 478
Hydrocyanic acid in flax, 66
Hymenochaete badio-ferruginea, 483; Mougeotii, 483; tabacina, 483
Hymenomycetous fungi of Siberia and eastern Asia—mostly of wood-destroying species, 469
Hypochnus spongiosus, 480; umbrinus, 480
Hysterangium, 457

I

igniarius (Fomes), 474
illaqueatum (Corticium), 484
inodora (Trametes), 474
Inonotus dryophilus, 336; *fibrillosus*, 334
investiens (Corticium), 484
Irpex, 334; fusco-violaceus, 478; *grossus*, 368; lacteus, 478; pachyodon, 479
Ischnoderma fuliginosum, 338; *resinosum*, 338

J

Juglandis (Microstroma), 486

K

Kneiffia setigera, 479

L

lacteus (Irpex), 478
lacteus (Polyporus), 473
lactescens (Corticium), 484
laevigata (Poria), 476
laricina (Lenzites), 470
Laubertia Boissieri, 556; **Pringlei**, 555; **Sanctae-Martae**, 555
laeve (Corticium), 484
lentus (Polyporus), 473
Lenzites, 390; abietina, 392; *abietinella*, 391, *452; abietis*, 328; heteromorpha, 470; *heteromorpha*, 366, 470; laricina, 470; *rhabarbarina*, 391; saepiaria, 391, *452; saepiaria porosa*, 370; septentrionalis, 470; serpens, 315; tenuis, 470; trabea, 392, *454; vialis*, 392
Lesquerella Kingii, 284; prostrata, 284
Leucocraspedum, 257; *albicaule*, 269; *albomarginatum*, 274; *coeruleum*, 271; *montanum*, 266
Leucogaster, 457; citrinus, 462; floccosus, 459; *luteomaculatus*, 462; nudus, 462
Light: effect of, on glucoside content of flax, 73; effect of, on growth and reproduction in plants, 573; ultra-violet, effect of measured intensities on behavior of plant cells, 489
Linamarine, 90
Linder, D. H. The genus Helicoceras, 1; Brief notes on the Helicosporeae, with descriptions of four new species, 9
Linkage between self-sterility allelomorphs and other genes, 108
Liverworts, fertilization in, 511
lumbricoides (Helicosporium), 10
lumbricopsis (Helicosporium), 10
luteo-alba (Femsjonia), 487
luteomaculatus (*Leucogaster*), 462

M

Macropharynx *fistulosa*, 552; **spectabilis**, 552
Macroscepis barbata, 560; **equatorialis**, 559
Malouetia **Killipii**, 551; pubescens, 552
Malva, 125; *malachroides*, 215, 217
Malvaceae, 124
Malvastrum 128; *confertum*, 213; spicatum, 128
Mandevilla, 556
Marsdenia **lauretiana**, 562; mollissima, 563
Melanogaster, 457
melanopus (Polyporus), 473
Mertensia incongruens, 286
Merulius, 322; *alveolaris*, 394; serpens, 477; tremellosus, 477
Mesadenia, 246, 257
Microstroma Juglandis, 486

Missouri soils, acidity of, 41
Mnium: effect of ultra-violet radiation on cell contents of leaves, 500; fertilization in, 510
Moisture: influence of, upon spore germination and growth, 300; soil, effect of, on growth of cotton plants, 578
Monilia Celtidis (Celtis), 2
Monilia Celtidis, 2, 3
Monochromatic ultra-violet light of measured intensities, Effect of, on behavior of plant cells, 489
Monochromator in ultra-violet radiation work, 496
Morgani (Helicoma), 13, 14
Mosses, fertilization in, 509
mucida (Poria), 476
Murashkinsky, K. E. Fungi received from, 469
Murashkinskyi (Hydnum), 477
Mougeotii (Hymenochaete), 483
mutata (Peniophora), 485
Myriadoporus adustus, 339

N

Nicotiana, self-sterility and self-fertility in, 97
Nicotiana alata, 97, × Langsdorfii, 97; Sanderae, 97
Nothoscordum bivalve, Chromosome complements of, 465
nudum (*Hydnangium*), 462
nudus (Leucogaster), 462
Nuttallia malvaeflora, 188
Nymphaearum (*Gyroceras*), 5
Nymphaearum (Helicoceras), 5, *8*
Nymphaearum (*Helicosporium*), 5

O

ochraceum (Corticium), 484
ochraceum (Hydnum), 478
ochroleucum (Stereum), 469, 481
Ochroporus odoratus, 370
Octaviania australiensis, 463; *Ravenelii*, 463; *rosea*, 462; *Stephensii*, 463, v. *Ravenelii*, 463
Octavianina Stephensii, 463
Odontadenia **affinis**, 549; **augusta**, 548; **cognata**, 546; coriacea, 551; Cururu, 549; geminata, 551; **glauca**, 550; hypoglauca, 551; **Killipii**, 546; nitida, 551; **Perrottetii**, 546; **polyneura**, 546, 551; **Sandwithiana**, 547; speciosa, 548; **stemmadeniaefolia**, 548
Odontia bicolor, 479
olivacea (Coniophora), 485
olivaceum (Helicoma), 11
Oryzae (Helicoceras), 6, *8*
osseus (Polyporus), 473

P

pachyodon (Irpex), 479
Palliseri (Fomes), 474
palmata (Thelephora), 479
Paramecia, effect of ultra-violet radiation on, 505
Paxina nigrella, 293
Pellia, fertilization in, 512, 530
Pelloporus perennis, 353
Peniophora corticalis, 484; *diffissa*, 485; gigantea, 485; mutata, 485; *quercina*, 484
pergamenus (Polystictus), 471
Phaeoporus applanatus, 374
Phaeolis sistotremoides, 347
Phaeostemma grandifolia, 561; **tigrina**, 560
Phellinus igniarius, 382; *isabellinus*, 363; *nigrolimitatus*, 380; *salicinus*, 384
Phellorinia, 459
Phlebia radiata, 479; strigoso-zonata, 479
Phosphorus content of higher plants, effect of ultra-violet radiation on, 565
phragmites (Helicosporium), 9
Physiology of plant disease, Studies in the, 57
Pini (Stereum), 482
pinicola (Fomes), 474
Placodes applanatus, 374; *fulvus*, 383; *pomaceus*, 383
Plantaginis (*Gyroceras*), 5
Plantaginis (Helicoceras), 5, *8*
plantaginis (Torula), 5
Pleurococcus, effect of ultra-violet radiation on cell contents of, 499
Pleurotus applicatus, 470
Podaxis, 459; anomalus, 459; Farlowii, 459, *464*
polygonium (Corticium), 484
Polyporaceae, 470; of Colorado, 287; factors determining the distribution of, 296
Polyporus, 317; abietinus, 328, *414;* adustus, 339, *420*, 471; alboluteus, 333, *416;* amorphus, 471; anceps, 343, *424; annosus*, 389; *applanatus*, 374; *arculariformis*, 346; arcularius, 346, *426; aurantiacus*, 334; benzoinus, 471; *benzoinus*, 338; borealis, 335, *418; Boucheanus peponinus*, 394; brumalis, 471; caesius, 337, *434; calceolus*, 358; *callosus*, 365; *caudicinus*, 356; *cervinus*, 367; cinnabarinus, 342, *426;* cinnamomeus, 354, *426;* circinatus, 349, *430; circumstans*, 388; *conchatus*, 384; confluens, 351, *434; corruscans*, 336; crispus, 340, *424;* cryptopus, 355, *428;* delectans, 472; *Demidoffii*, 385; dichrous, 472; dryadeus, 472; *dryophilus*, 336; *dualis*, 349; elegans, 359, *432;*

Ellisianus, 343; *ferruginosus*, 401; fibrillosus, 316, 334, *412*, 472; *Flabellum*, 329; fragilis, 341, *426;* frondosus, 472; *fuliginosus*, 338; *fulvus*, 338; *Gillotii*, 389; gilvus, 472; *hirsutulus*, 323; hirsutus, 324, *412;* hirtus, 357, *432; hispidellus*, 357; *hispidoides*, 347; hispidus, 472; *hyperboreus*, 382; *igniarius*, 382; *ilicincola*, 330; *inflatus*, 321; *irregularis*, 389; *Juniperinus*, 385; *laceratus*, 329; lacteus, 473; *laetificus*, 400; lentus, 473; leucomelas, 288; *leucophaeus*, 374; leucospongia, 326, *412; Lindheimeri*, 368; *Lloydii*, 326; *loricatus*, 384; *Macouni*, 401; *marginatus*, 377; *medulla-panis*, 398; *megaloma*, 374; melanopus, 473; *Menandianus*, 329; mollis, 342; *nigricans*, 382; *nigrolimitatus*, 380; *Novae-Angliae*, 382; *oblectans*, 354; *obvolutus*, 321; *odoratus*, 370; osseus, 360, 473; ovinus, 350, *426; pallescens*, 339; pargamenus, 329, *410; parvulus*, 328, 354; *Peakensis*, 349; perennis, 353, *430; piceinus*, 379; picipes, 359; *Pini*, 379; *pinicola*, 377; planellus, 344, *422; planus*, 344; *pomaceus*, 383; *prolificans*, 329; *pseudopargamenus*, 330; pubescens, 326; *purpurea*, 401; resinosus, 338, 389, *422*, 473; Rheades, 336, *420; roseus*, 387; *rufo-pallidus*, 387; *salicinus*, 384; *salmonicolor*, 400; *Sartwellii*, 330; *scalaris*, 365; Schweinitzii, 347, *428; serialis*, 365; *serpentarius*, 389; *Shiraianus*, 334; *spectabilis*, 347; *spissus*, 400; splendens, 354; spumeus, 345, *434*, 473; squamosus, 356, 473; *steroides*, 367; *subacida*, 399; subchartaceus, 331, *414; subcinereus*, 339; *subflavus*, 330; *subpileatus*, 389; *subsericeus*, 354; subsquamosus, 356; *tabulaeformis*, 347; *tenuis*, 363; *tomentosus*, 349; trichrous, 473; *ursinus*, 332, *416; vaporaria*, 397; *variiformis*, 365; varius, 358, *432;* versicolor, 323, *410;* volvatus, 321, *410; volvatus Helix*, 321; *zalapensis*, 330; *zantholoma*, 398; *Zelleri*, 360; zonatus, 325, *410*

polysporum (Helicoma), 11

Polystictus, 313; *abietinus*, 328, 470; *azureus*, 323; *biformis*, 471; *Celottianus*, 368; *cinnabarinus*, 342; *cinnamomeus*, 354; *Fergussoni*, 368; *hirsutus*, 324, 471; *hirtellus*, 324; *pargamenus*, (*pergamenus*), 330; *perennis*, 353; pergamenus, 471; *piceinus*, 379; *pusio*, 328; radiatus, 471; *Schweinitzii*, 347; *scuirinus*, 368; *subchartaceus*, 331; *versicolor*, 323; vulpinus, 471; *zonatus*, 326

Poria, 313, 395; caesio-alba, 476; *callosa*, 365, 396; contigua, 364; ferruginosa, 364, 401; *incerta*, 397; *laetifica*, 400; laevigata, 476; *Macouni*, 401; medullapanis, 398, *456;* monticola, 396, *454;* mucida, 398, 476; *obducens*, 396; purpurea, 401; *salmonicolor*, 400; spissa, 400, *456;* subacida, 399, *456;* superficialis, 364; taxicola, 476; Vaillantii, 398; vaporaria, 397, *456;* violacea, 401; viticola, 364; xantha, 476; *zantholoma*, 398

Porodaedalea Pini, 379

Porothelium Friesii, 476

Prestonia acutifolia, 554; **agglutinata**, 552; **coalita**, 552; **Dusenii**, 552; **isthmica**, 555; *Langlassei*, 555; *macrocarpa*, 553; mexicana, 555; Muelleri, 553; **portobellensis**, 553; Riedelii, 553; **velutina**, 554

protracta (Trametes), 475

pubescens (Polyporus), 473

Pycnoporellus fibrillosus, 334

Pycnoporus cinnabarinus, 342

Pyropolyporus conchatus, 384; *Earlei*, 385; *fulvus*, 383; *Juniperinus*, 385

Q

Quartz mercury vapor arc, Stimulatory effects of radiation from, 17

quercina (Daedalea), 476

quercina (*Peniophora*), 484

Quinhydrone electrode method for testing soils, 41

R

radiata (Phlebia), 479

radiata (Trametes), 475

Radiation: Stimulatory effects of, from a quartz mercury vapor arc, 17; ultra-violet, effect of, upon plants, 489, upon the calcium and phosphorus content of higher plants, 565

radiatus (Polystictus), 471

radiosum (Corticium), 484

Rauwolfia bahiensis, 543; **lauretiana**, 541; paucifolia, 542; **sanctorum**, 542

Ravenelii (Arcangeliella), 463

Ravenelii (*Hydnangium*), 463

Ravenelii (*Octaviania*), 463

reflexum (Hydnum), 478

repens (Helicoma), 11

resinosus (Polyporus), 473

Reynolds, Ernest S. Studies on the physiology of plant disease, 57

Rhabdadenia polymeura, 546

Rhaptocarpus coalitus, 552

Rhizopogon, 457

Rhoeo discolor, effects of ultra-violet radiation on cell contents of, 499

rhytidocyclum (Stereum), 482

Riccardia, fertilization in, 512, 530
Rocky Mountain plants, Notes on the distribution of some, 283
rosea (*Octaviania*), 462
rosea (Arcangeliella), 462
roseum (Corticium), 484
roseus (Fomes), 474
roseus (Helicomyces), 10, 16
Roush, Eva M. Fling. A monograph of the genus Sidalcea, 117
rugosiusculum (Stereum), 482
rugosum (Stereum), 482
rutilans (Polyporus), 473

S

salicina (Cytidia), 486
sanguinolentum (Stereum), 482
Sarcopodium, 2
Sarcopodium (Tricholeconium) **ammonis**, 7
saxonicum (Coremiella), 7
saxonicum (*Gyroceras*), 7
Schizophyllum commune, 470
Scutiger cryptopus, 355; *hispidellus*, 357; *laeticolor*, 352; *ovinus*, 350; *Whiteae*, 352
Sebacina calcea, 487
Self-sterility and self-fertility in Nicotiana, Genetic analysis of an unusual relationship between, 97
septentrionalis (Lenzites), 470
Septobasidium Carestianum, 487
serpens (Merulius), 477
Sesia hirsuta, 391; *pallidofulva*, 393
setigera (Kneiffia), 479
Shope, Paul Franklin. The Polyporaceae of Colorado, 287
Siberia, Hymenomycetous fungi of, 469
sibirica (Coniophora), 485
Sida, 117, 125, 197; *californica*, 199; *delphinifolia*, 143, 145, 188, 193, 197; *diploscypha*, 139, *heterophylla*, 173; *malvaeflora*, 175, 188; *oregana*, 168
Sidalcea, A monograph of the genus, 117
Sidalcea, 117, 136; sect. **Annuae**, 138; *asplenifolia*, 175, 177; asprella, 201; *atacosa*, 218; atropurpurea, 218; *californica*, 199; calycosa, 145, *230;* campestris, 175; *campestris*, 169, 218; candida, 153, *228*, *234;* *candida* var. *tincta*, 153; *confinis*, 179; *Covillei*, 187; *crenulata*, 179; *Cusickii*, 174; *delphinifolia*, 188, var. *humilis*, 189; diploscypha, 139, *228*, *232;* *diploscypha* var. *minor*, 139; *elegans*, 202; subgenus Eusidalcea, 129, 138; *eximia*, 159; *favosa*, 200; glaucescens, 207; Hartwegi, 148, *230;* *Hartwegi* var. *tenella*, 148; subgenus Hesperalcea, 128, 214; Hendersoni, 157; Hickmani, 212; Hickmani var. Parishii, 213, *228*, *230*, *244;* *Hickmani*, 213; hirsuta, 143, *230;* *humilis*, 188; *hydrophila*, 159; *interrupta*, 166; malachroides, 215, *228*, *230*, *244;* malvaeflora, 188, *228*, *240*, var. atropurpurea, 218, var. californica, 199, var. Listeri, 218; *malvaeflora*, 157, 168, 179, var. *asprella*, 202, var. *Oregana*, 157, 168; subgenus **Malvastralcea**, 128, 212; mariana, 218; mexicana, 218; *montana*, 207; multifida, 209, *240;* "*Murrayana*," 159; *Nelsoniana*, 159; neo-mexicana, 179, var. **Covillei**, 187, var. **parviflora**, 186; *neo-mexicana* var. *Diehlii*, 179; *nervata*, 169; *nitrophila*, 186; nodosa, 218; oregana, 168, 177, *230*, *236*, var. **Cusickii**, 174; *oregana*, 175; *Parishii*, 213; *parviflora*, 186, var. *Thurberi*, 179; pedata, 210, *242;* sect. **Perennes**, 151; peruviana, 218; *ranunculacea*, 166; reptans, 200; *rhizomata*, 145; **robusta**, 205; *rostrata*, 189; *scabra*, 189; *secundiflora*, 139; spicata, 159, var. **ranunculacea**, 166, *230*, *238;* *spicata* var. *pedata*, 210, var. *reptans*, 200; *sulcata*, 145; *sylvestris*, 175; *tenella*, 148; triloba, 218; *valida*, 159; virgata, 178; *vitifolia*, 215
Sidalcia, 145
Soils in Missouri, the acidity of, and its relation to plant distribution, 41
spadiceum (Stereum), 483
Sphaeralcea, 213; pedatifida, 218
Sphaerocarpos, fertilization in, 511, 530
spinulosa (Eichleriella), 487
Spongipellis borealis, 335; *fragilis*, 341; *occidentalis*, 345; *spumeus*, 345
spongiosus (Hypochnus), 480
Spongiporus leucospongia, 326
spumeus (Polyporus), 473
squamosus (Polyporus), 473
Stephanotella Glaziovii, 558; **Killipii**, 558
Stephensii (Arcangeliella), 463
Stephensii (*Hydnangium*), 463
Stephensii (*Octaviania*), 463
Stephensii v. *Ravenelii* (*Hydnangium*), 463
Stephensii (*Octavianina*), 463
stereoides (Trametes), 475
Stereum bicolor, 481; Chailletii, 480; fasciatum, 480; fuscum, 481; gausapatum, 481; hirsutum, 481; ochroleucum, 469, 481; Pini, 482; rhytidocyclum, 482; rugosiusculum, 482; rugisporum, 292; rugosum, 482; sanguinolentum, 482; spadiceum, 483; sulcatum, 483; versiforme, 483
Steyermark, Julian A. A study of plant distribution in relation to the acidity of various soils in Missouri, 41

Stimulatory effects of radiation from a quartz mercury vapor arc upon higher plants, 17
Streptotrachelus Pringlei, 555
Strilia cinnamomea, 354
Studies on the physiology of plant disease, 57
suaveolens (Trametes), 475
sulcatum (Stereum), 483
sulphureum (Corticium), 484
Sweertia, 257; *albicaulis*, 269; *albomarginata*, 274; *Bigelowii*, 279; *carolinensis*, 262; *fastigiata*, 264; *Parryi*, 273; *radiata*, 259
Swertia, 245, 247
Swertia, 257; *albomarginata*, 274; *difformis*, 262; *fastigiata*, 264; *Frasera*, 262; *neglecta*, 263; *nitida*, 265; *Parryi*, 273; *tubulosa*, 271

T

tabacina (Hymenochaete), 483
Tabernaemontana hirtula, 541; **Killipii**, 541
taxicola (Poria), 476
Temperature: effect of, on glucoside content of flax, 73; effect of variations in, and lengths of day, on vegetative growth and reproduction in cotton, 573; influence of, upon spore germination and growth, 297
tenuifilum (Helicoma), 14, *16*
tenuis (Lenzites), 470
tenuis (Thelephora), 479
terrestris (Thelephora), 480
Tessaranthium, 257; *angustifolium*, 259; *macrophyllum*, 259; *scabrum*, 259; *speciosum*, 259; *stenosepalum*, 259
Tesseranthium, 246, 257; *radiatum*, 259
Thelephora palmata, 479; **tenuis**, 479; terrestris, 480
Thelephoraceae, 479
Tilsit soils in Missouri, 42
Tomatoes, effect of ultra-violet radiation: on growth, 28, 36, 568; on calcium content, 570; on phosphorus content, 569
Torula Plantaginis, 5
Tradescantia reflexa, effect of ultra-violet radiation on cell contents of stamen hairs, 499
Trametes, 362; Abietis, 474; *benzoina*, 338; *carnea*, 372; *cinnabarina*, 342; gallica, 369; gibbosa, 475; heteromorpha, 366, *440*; hispida, 368, *440*, 474; inodora, 474; isabellina, 363, *438*; *laceratus*, 366; *mollis*, 367; odorata, 370, *440*; *Peckii*, 368; *Pini*, 379; *Pini Abietis*, 379; *protracta*, 370, 475; **radiata**, 475; *radiciperda*, 389; serialis, 365, *438*; *setosus*, 363; stereoides, 367, *440*, var. Kmetii, 475; stuppeus, 370; suaveolens, 475; subrosea, 372, *442*; *tenuis*, 363; *trabea*, 475; variiformis, 365, *438*
tremellosus (Merulius), 477
Tremellaceae, 487
Tricholeconium, 2
trichrous (Polyporus), 473
Tyromyces anceps, 343; *caesius*, 337; *Ellisianus*, 343

U

Ultra-violet light, Effect of monochromatic, of measured intensities, on behavior of plant cells, 489
Ultra-violet radiation: effects of, upon plants, 17, 489; Some effects of, upon calcium and phosphorus content of higher plants, 565
umbrinus (Hypochnus), 480
Ungulina annosa, 389; *fuliginosa*, 338; *marginata*, 377; *rosea*, 387; *volvata*, 321, var. *pleurostoma*, 321
unicolor (Daedalea), 476
Union soils in Missouri, 47

V

velutinum (Hydnum), 478
versiforme (Stereum), 483
Villarsia pumila, 286
violaceum (Helicoma), 14
virescens (*Hydnangium*), 462
Vita-glass filter in ultra-violet radiation experiments, 30
vulpinus (Polystictus), 471

W

Westoni (Helicoma), 12, *16*
Wherry's soil acidity results, compared with Steyermark's, 51
Woodson, Robert E. Jr. New or otherwise noteworthy Apocynaceae of tropical America, 541; New South American Asclepiadaceae, 557
Wynd, F. Lyle, and Harry J. Fuller. Some effects of ultra-violet radiation upon the calcium and phosphorus content of higher plants, 565

X

xantha (Poria), 476

Z

Zea Mays, effect of ultra-violet radiation on, 566

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